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THE BOTANICAL GAZETTE

EDITOR
E. J. KRAUS

VOLUME 100

WITH SIX HUNDRED AND TWENTY-FOUR FIGURES



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THE BOTANICAL GAZETTE

September 1938

COMPARATIVE ANATOMY OF THE MORACEAE AND THEIR PRESUMED ALLIES¹

OSWALD TIPPO

(WITH SIXTY-THREE FIGURES)

Introduction

It has become increasingly evident that if botanists are ever to establish a natural classification of the angiosperms, this natural system will be the combined work of taxonomists, anatomists, cytologists, palcobotanists, and specialists in related fields of research. Most of the present day systems are based on the morphology of the flower, and to a lesser extent on the general external structure of the plant body. Even so, the arrangements of the plant groups are many and various. This multiplicity of systems is largely due to the lack of agreement as to what constitutes a primitive angiosperm. This in turn is traceable to the dearth of fossil evidence on the early history of the flowering plants. Consequently the concept of the primitive angiosperm varies with the individual phylogenetist, depending upon the group of gymnosperms which is selected as the progenitor of the angiosperms. Next, various attempts have been made to establish criteria or dicta of evolution, such as BESSEY'S Dicta (33), HUTCHINSON'S General Principles (83), and ENGLER'S Prinzipien (62). Finally, the groups of plants have been arranged phylogenetically according to the dicta or principles of the individual creating the system.

¹ Contribution from the Biological Laboratories, Harvard University.

ENGLER and PRANTL (60, 61, 62) derive the Verticillatae (Casuarinaceae), the Juglandales (Juglandaceae), the Fagales (Betulaceae and Fagaceae), the Urticales (Ulmaceae, Rhoipteleaceae, Moraceae, and Urticaceae), and the Ranales in independent lines from a hypothetical group, the Protangiospermae. The Rosales are then shown as coming from the Ranales. In the Urticales, the Ulmaceae are considered to be primitive.

WETTSTEIN (158) derives the Verticillatae from the Gymnospermae. The Fagales and the Juglandales are considered to be rather closely related and the two groups have their origin in the Gymnospermae. The Urticales (Moraceae, Cannabaceae, Ulmaceae, Eucommiaceae, Rhoipteleaceae, and Urticaceae) are derived from the same line which produced the Fagales and the Juglandales. The Hamamelidales arose close to the Urticales. The Rosales are connected up to the former group through the Polycarpicae.

HUTCHINSON (83), in his phylogenetic arrangement of the dicotyledons, pictures evolution as proceeding from the Magnoliales, to the Dilleniales, to the Cunoniales, to the Rosales, and then to the Hamamelidales. The Fagales, the Casuarinales, and the Urticales (Ulmaceae, Barbeyaceae, Moraceae, Scyphostegiaceae, Urticaceae, and Cannabinaceae) are derived in independent lines from the Hamamelidales. However, some of the Urticales are shown as taking their origin from his herbaceous line. These herbaceous Urticales are connected up with the Aristolochiales, which in turn arose from the primitive Ranales through the Berberidales.

BESSEY (31, 32, 33) places the Ulmaceae, the Moraceae, and the Urticaceae in the Malvales. These three families are derived from the Tiliaceae. The Malvales originated from the Ranales. The Rosales also are shown as taking their origin in the Ranales. In the former order the Rosaceae are considered to have given rise to the Hamamelidaceae and allied families. The Casuarinaceae are joined up with the Hamamelidaceae. The Sapindales, according to BESSEY, had their origin in the Rosales through the Celastrales. The Sapindaceae are figured as giving rise to the Juglandaceae on the one hand, and to the Betulaceae and Fagaceae on the other.

HALLIER (79) derives the Hamamelidales from the Ranales through the intermediate group, the Anonales. The Terebinthales are derived from the Ranales. In the Terebinthales, the Rutaceae have given rise to the Terebinthaceae (including the Juglandaceae). The Terebinthaceae have given rise to the Urticaceae (broad sense) on the one hand, and to the Betulaceae, Fagaceae, and Casuarinaceae on the other hand. The Rosales are derived from the Ranales on still another line.

In addition to these great systems of classification, mention may be made of some of the systems of lesser prominence. RENDLE (125) follows ENGLER and PRANTL in the main. The Juglandales, Fagales, Casuarinales, Urticiflorae, and other Amentiferae are placed in a low position. The Urticiflorae are made up of the following families: Ulmaceae, Urticaceae, Moraceae, and Cannabinaceae. JOHNSON (91) also follows the Englerian scheme for the most part. He groups the families Ulmaceae, Moraceae, Urticaceae, and Cannabinaceae in the order Urticales. BENTHAM and HOOKER (21) place the Urticaceae (broad sense) in the artificial series Unisexuales, along with the Euphorbiaceae, Balanopseae, Platanaceae, Leitnerieae, Juglandaceae, Myricaceae, Casuarineae, and Cupuliferae. WARMING and MÖBRUS (150) begin their classification of the dicotyledons with the orders Verticillatae, Querciflorae, Juglandiflorae, Saliciflorae, and Urticiflorae. The latter order contains the Ulmaceae, Urticaceae, Moraceae, and Cannabinaceae. Unfortunately, LOTSY did not reach the Urticales in his unfinished work (105).

It is apparent that there is a wide difference of opinion among the taxonomists as to the proper phylogenetic position of the Moraceae. Since this is the situation, it is only logical that the student of phylogeny should turn to explore the evidence from the other divisions of botanical science in his attempt to determine which, if any, of the existing phylogenetic systems is the more natural arrangement of the flowering plants.

The present study is an investigation of the anatomy of the Moraceae and of the several families which have been placed near them in the different systems of phylogeny. The attempt is made to use this anatomical evidence in tracing the phylogeny of this group of families. Further, there has been an endeavor to harmonize

the anatomical data with the facts of floral morphology and with the evidence from the other branches of plant science, such as cytology, paleobotany, and floral anatomy.

The anatomists have established, entirely independently of taxonomy and of systems of phylogeny, certain lines of specialization or trends of evolution in the structures of the plant stele. It is held by many anatomists (135, 136, 63, 132, 120, 121, 159, 87, 109, 151, 108, 1, 148, 72, 73, 74) that these evolutionary tendencies in the internal structure of plants should be of some aid to taxonomy and to phylogeny. Further, BAILEY and SINNOTT (16) and BAILEY (11) have pointed out the necessity for the study of all parts of the plant before a natural classification can be formulated. It must be made clear, however, that the anatomist is not striving to base classification on anatomical features alone. He does not argue that the anatomy of plants is necessarily more conservative than the structure of the flower or fruit. He recognizes that in evolution not all the structures of an organism may be proceeding in specialization at the same rate; thus in some groups the flower probably has evolved relatively rapidly, with the stem remaining more or less at the same evolutionary level, while in other groups the stem has become specialized with the reproductive organs remaining more or less the same. But the anatomist does feel that any truly natural classification must take cognizance of the facts of internal structure as well as those of external morphology.

These lines of structural evolution, chiefly in the secondary xylem, may be summarized briefly as follows:

1. The vessel element with a scalariform perforation plate (fig. 28) is more primitive than the element with a simple perforation plate (fig. 6) (87, 18, 141, 142, 34, 64, 65).

2. Vessel elements with scalariform perforation plates may have apertures with complete borders, with borders to the middle of the orifice, with borders only at the ends of the orifice, and with non-bordered apertures (fig. 28). Evolution has proceeded in the order just given (65).

3. In the class of vessel elements with scalariform perforation plates, the type with many (over 15) bars to a plate (fig. 57) is primitive; the type with intermediate (5-15) bars (fig. 28) comes next; and the type with few (5 or fewer) bars is highest (fig. 61) (65).

4. Among the vessel elements with scalariform perforation plates, the type with narrow openings (fig. 57) precedes the type with wide openings (65).

5. There is a decrease in length of vessel element as the vessel elements become specialized (fig. 23) (18, 9, 64).

The length of a vessel element is determined by two factors: (1) the length of the cambial initial from which it was derived, and (2) the amount of elongation which takes place during differentiation. BAILEY (9) has shown that as the secondary xylem of the gymnosperms and angiosperms becomes more specialized, the cambial initials decrease in length. Further, he demonstrated that in most cases the vessel members are equal to the cambial initials in length. The latter conclusion was also reached by CHALK and CHATTAWAY (44). However, BAILEY (9) did find that some of the more primitive types of vessel elements are slightly longer than their cambial initials, and some of the more advanced types of vessel members are slightly shorter than their cambial initials. Likewise CHALK and CHATTAWAY (45) report that the vessel elements in the early wood of certain ring-porous woods are considerably shorter than their cambial initials. It is clear, therefore, that the fact that the vessel elements show a gradual decrease in length in the dicotyledons is dependent on the fact that the cambial initials are decreasing in length as the woods of these plants become more specialized. The two exceptions just noted merely serve to accentuate the effects of this shortening process at the two ends of the series.

6. There is an increase in the diameter of the vessel elements as they become specialized (figs. 38, 19, 3) (18, 9, 64).

7. Vessel members with angular cross-sectional outline (fig. 39) are primitive; those with round outlines (fig. 26) are derived (11, 64).

8. Vessel elements with thin walls (fig. 39) precede those with thick walls (fig. 19) (64).

9. As the vessel elements become specialized, their end walls become less and less oblique until a transverse end wall is produced (figs. 57, 23) (18, 64, 65).

10. The evolutionary development of intervacular pitting and vessel-parenchyma pitting proceeds from scalariform (fig. 55) to transitional (fig. 54) to opposite (fig. 53) to alternate (fig. 6) (38, 18, 64, 66).

11. Diffuse-porous woods (fig. 4) precede ring-porous woods (fig. 3) (64).

12. Spiral thickenings in vessel members are evidence of specialization (66).

13. The solitary pore arrangement (fig. 39) is more primitive than the various aggregate arrangements of pores; that is, pore multiples (fig. 15), pore clusters (fig. 18), and pore chains (FROST, unpublished data).

14. Evolution has proceeded from tracheids (fig. 52) to fiber-tracheids (fig. 49) to libriform wood fibers (fig. 56) (87, 18, 11, 13).

15. There is a phylogenetic decrease in length in the fibrous tracheary elements as the tracheids, fiber-tracheids, and libriform wood fibers become more and more specialized (18, 9, 11).

As with the vessel elements, the length of fibrous tracheary elements is determined by two factors: (1) the length of the cambial initials from which they were derived, and (2) the amount of elongation which takes place during differentiation. It has already been stated that the length of the cambial initials decreases as specialization proceeds in the secondary xylem of the dicotyledons. CHIATTAWAY (48) has found that the percentage of elongation of the fibers increases as the cambial initials become shorter. In other words, very long cambial initials do not elongate much in the ontogenetic differentiation of fibers, but short cambial initials may elongate as much as nine times their original length in the formation of mature fibers. Yet the amount of decrease in the length of the cambial initials is so much greater than the amount of elongation that the generalization that fibrous tracheary elements decrease in length with specialization of the wood is not upset.

16. The non-septate fiber, either fiber-tracheid or libriform wood fiber, precedes the septate fiber, either septate fiber-tracheid (fig. 2) or septate (libriform) wood fiber.

17. Heterogeneous rays are more primitive than homogeneous rays. KRIBS (102) has shown that the evolutionary sequence is from heterogeneous type I, the most primitive, through the transitional heterogeneous types IIA (fig. 7) and IIB (fig. 8), to homogeneous type I (fig. 10), to the highly specialized homogeneous type II rays. Further, the uniseriate homogeneous type III rays (fig. 32) are more

highly specialized than the uniseriate heterogeneous type III rays (fig. 9). Heterogeneous type III is regarded as a derivative of either heterogeneous type I or II. Homogeneous type III may have evolved from any of the other types. It appears that the uniseriate types are highly specialized structures, owing to the elimination of multiseriate rays.

The status of the so-called aggregate, diffuse, and compound rays is such a controversial matter that no final statement concerning them can be made at the present time. However KRIBS (102), after an extensive survey of rays in the dicotyledons, comes to the conclusion that "the so-called compound ray is merely an unusually wide multiseriate ray. Such rays are of common occurrence in both primitive and specialized types of dicotyledons, and may be of either the heterogeneous or the homogeneous type. The so-called aggregate ray is a specialization which occurs sporadically. It is an offshoot from the main line of structural specialization in rays."

18. As for xylem parenchyma, the diffuse type (fig. 38) is primitive and the various aggregations known as vasicentric (fig. 22), aliform (fig. 11), confluent (fig. 19), and metatracheal parenchyma (fig. 15) are derived (BAILEY, unpublished data; 87).

19. BAILEY (10, 11) has shown that in structurally primitive woods, the fusiform initials of the cambium overlap and the increase in the circumference of the lateral meristem is due to pseudo-transverse, anticlinal divisions followed by longitudinal sliding growth of these cells. Consequently the cells of the mature wood are non-stratified. This type of cambial activity culminates in a type where the anticlinal divisions of the fusiform initials are radio-longitudinal. In such stems the cambial derivatives are arranged in parallel horizontal series. Thus the storied arrangement of elements (fig. 23); that is, rays, vessel members, and even fibers and wood parenchyma strands, indicates a highly specialized stem (116, 119, 85).

20. Herbs have been derived from shrubs and trees (57, 133, 134, 87, 89, 90).

It will serve a useful purpose to refer briefly to the methods by which these salient lines of structural specialization have been worked out. In 1918, BAILEY and TUPPER (18) made an extensive

survey of the lengths of vessel elements and other tracheary cells in a great many living gymnosperms and dicotyledons, as well as a goodly representation of fossil Lycopsida, Sphenopsida, and Gymnospermae. They concluded that, in general, there is a tendency for the reduction in length of the tracheary cells (vessel elements, tracheids, fiber-tracheids, and libriform wood fibers), proceeding from the lower groups such as the Lycopsida, Sphenopsida, and Cycadofilicales, to the Cordaitales, Bennettitales, and Cycadales, to the Coniferales and Gnetales, and thence to the dicotyledons. This decrease in length continues in the dicotyledons. Further, the evolutionary specialization of the vessel element proceeds hand in hand with this reduction in length of the tracheary cells. That is, a phylogenetic study indicates that as the tracheary elements become shorter and shorter, the vessel members pass from the scalariform to the simple perforate state, they become wider, the pitting on their lateral walls changes from scalariform to transitional to opposite to alternate, and their end walls become less and less tapering. That the appearance and subsequent evolution of the vessel are correlated with a decrease in cell size is emphasized by the fact that the vesselless angiosperms, *Trochodendron* and *Drimys*, have the longest tracheary elements of any of the dicotyledons investigated by BAILEY and TUPPER. Furthermore, the tracheary elements of the Gnetales which possess vessels resemble in size those of many dicotyledons. Finally, correlated with this decrease in size and with this vessel evolution, the fibrous tracheary elements become increasingly specialized; that is, the bordered pits become smaller and smaller, until ultimately a libriform wood fiber with simple pits is produced.

FROST and KRIBS, using the research of BAILEY and TUPPER as a basis, found that the specialization of other features of the vessel members and of the rays correlated to a remarkable degree with the decrease in size of the tracheary cells.

From this brief description of the methods of the anatomist, it can be seen that these lines of specialization are not based on any system of angiosperm classification, but have been formulated independent of any preconceived notion that this or that group of gymnosperms has given rise to the angiosperms; or any preconceived idea that the Ranales or the Amentiferae are primitive. Con-

sequently any suggestions which the anatomist makes as to phylogeny will be unbiased, and therefore of correspondingly greater value than would be the case were not his methods founded independent of classification.

Material and methods

Slides of the secondary xylem from the stems of 465 species, representing 165 genera and 22 families, were examined in detail during this investigation. These families (defined by ENGLER and PRANTL (61, 62) unless otherwise indicated) include the Moraceae, Ulmaceae, Urticaceae, Rhoipteleaceae (60), Hamamelidaceae, Eucommiaceae, Platanaceae, Myrothamnaceae, Buxaceae, Stachyuraceae, Casuarinaceae, Betulaceae, Fagaceae, Juglandaceae, Hydrangeaceae (family by HUTCHINSON, 83; equivalent to the subfamily Hydrangeoideae of the Saxifragaceae of ENGLER and PRANTL), Grossulariaceae (family by HUTCHINSON, 83; equivalent to the subfamily Ribesioideae of the Saxifragaceae of ENGLER and DIELS, 60), Escalloniaceae (family by Hutchinson, 83; equivalent to the subfamily Escallonioidae² in the Saxifragaceae of ENGLER and PRANTL, 61), Brunelliaceae, Cunoniaceae, Dichapetalaceae (or Chailletiaceae), Rosaceae, and Calycanthaceae. In addition, all available slides of the Aristolochiaceae, Sterculiaceae, and Tiliaceae were studied, but no detailed descriptions were made of these groups since anatomically they were found not to be closely related to the Moraceae.

The material of the Moraceae was collected in various parts of the world by many different collectors. The major part of the collection was assembled at the Yale School of Forestry. It was through the courtesy of Professor SAMUEL J. RECORD of Yale University that most of the woods were made available for the present study.

Following the technique outlined by BAILEY (4), JEFFREY (88), and WETMORE (156), small blocks of the woods were boiled, cooled, and then softened in hydrofluoric acid. Next the material was washed, dehydrated, and stored in glycerin-alcohol. Later transverse, radial, and tangential sections were cut with a Jeffre-

² In the second edition of ENGLER and PRANTL (62), four other sub-families are split off from this one sub-family.

Thomson sliding microtome. These sections, 10–15 μ in thickness, were stained in Heidenhain's iron-alum haematoxylin and then counter-stained in safranin.

The xylem of the other families was studied from slides in the extensive Harvard collection.

Eight or nine slides of each species were available in the material of the Moraceae. Often two or more representatives of the same species from different geographical localities were studied. In the other families, more than one slide of each species was studied whenever possible, and frequently specimens of the same species from different parts of the world were examined microscopically.

The terms used in the anatomical descriptions of the families which follow this section are those approved by the Committee on Nomenclature of the International Association of Wood Anatomists (50). These terms have been further elaborated and illustrated by RECORD, the chairman of the Committee (119). After the appearance of these two publications on terminology, BAILEY (13) gave more practical definitions of the terms tracheid and fiber-tracheid. His usage of the two terms is followed in this study; that is, fiber-tracheids are elements with pits with much reduced or vestigial borders—conspicuously smaller than the corresponding pits on the vessel members (fig. 49)—and tracheids are elements with large bordered pits (equal in size to the pits on the vessel elements) (*cf.* figs. 52 and 53).

FROST (65) has been followed in classifying scalariform perforation plates as to the number of bars; that is, the plates with 15 bars or more are placed in the many class, those with 5 to 15 bars are placed in the intermediate class, and the plates with 5 or fewer bars in the few class. Further, the scalariform perforation plates with openings of 6 μ (measured from bar to bar) or less are described as narrow; those with apertures of more than 6 μ are designated wide.

KRIBS' (102) classification of rays is used in this investigation. Ray width and ray depth do not appear to have much phylogenetic significance; indeed it is questionable whether they are of any great diagnostic value, for they exhibit considerable variation, not only from tree to tree, depending on site, but at different levels in the same tree, and at various distances from the pith at the same level (8, 111, 55, 152). However, a brief notation on the range of varia-

tion in ray width and depth is included in the anatomical description of each family.

Whenever the anatomical description of a family was, by necessity, based on material cut from a region close to the pith, this fact is indicated. The ideal situation, of course, would be one where it would be possible always to compare the homologous parts of the plants of all the families, for it has been shown by BAILEY and FAULL (14) that in a given species the range of variability tends to be greater in different parts of a single, large mature tree than in homologous parts of different trees. Since this ideal arrangement is not always possible to attain, the next best thing is to record any youthful material employed in an investigation so that a correction may be made in the interpretation of any conclusions based on this young material. Many investigators (130, 15, 115, 18, 101, 42) have shown that from the pith outward, the lengths of vessel elements and of other tracheary cells increase very rapidly for a number of years, and then remain at about the same relative length, but fluctuating slightly with changes in environmental conditions. Vessel diameter also increases from the pith out to the bark (115, 9). Multiseriate ray width increases centrifugally from the pith (55). Multiseriate ray depth seems to decrease from the pith outward (BAILEY, unpublished data). Further, plants with homogeneous rays in the mature wood may have heterogeneous rays in the wood near the pith (figs. 33-36). Thus it seems that there is an increase in the homogeneity of the rays from the pith out to the cambium. Enough has been said about variation from youth to old age in wood to demonstrate the importance of using homologous regions in comparative anatomical studies. At least, if this ideal is not always attainable, any departures from the use of homologous regions should be recorded.

Rough measurements of vessel diameter and vessel element length are given for each family. These figures were derived in the following manner: Only one species of each genus was measured. For the vessel diameter (tangential) figures, 25 vessels, selected at random, were measured from sections with a graduated ocular disc calibrated against a stage micrometer. These measurements were averaged and the resulting mean was then averaged with the means of other genera in computing the mean for the family. The measurements of vessel element length were treated in the same way, except

that only 12 vessel members were measured from sections. The total body length of the vessel element (that is, the extreme tip of one end to the tip of the other) was measured, for it has been shown that total body length, rather than body length or extreme body length, corresponds more nearly to the length of the cambial initial from which the vessel element was derived (44). In ring-porous woods the elements in the late wood were measured, for their length is nearer that of the cambial initials than are the vessel members in the early wood (45). During differentiation the vessel elements in the latter region undergo considerable shortening, probably owing to the great lateral swelling of the vessel members in this zone. CHALK (43) has set up certain size classes for vessel element length, and in recording the present measurements, the size classes are indicated. These size classes are defined as follows:

SHORT	
Extremely short	less than 177 μ
Very short	177-240
Moderately short	240-342
MEDIUM SIZED	
Medium sized	342-790
LONG	
Moderately long	790-1131
Very long	1131-1866
Extremely long	over 1866

The size classes employed for the vessel diameter figures are those of CHATTAWAY (47). The limits of these classes are as follows:

Extremely small	up to 30 μ
Very small	30-50
Small	50-100
Moderate sized	100-200
Rather large	200-300
Large	300-400
Very large	over 400

Of course these measurements on the vessel elements are of the crudest sort. In the first place, the method of measuring vessel element length on sections gives only approximate lengths, for often the "tails" or "ligules" of vessel members elude the observer.

Macerated material should be used for best results. In the second place, too few measurements for each species were made. In the third place, these figures in no way represent the mean for the species, for it has been shown by RENDLE and CLARKE (126, 127) that the variations in cell size in wood are of 4 types: (a) Local variation in a small sample owing to differences in development of the various cells from the cambium. (b) Variations from the pith to the periphery. (c) Variations from the base of the stem upward (130, 42, 54). (d) Variations resulting from differences in external growth conditions (113). Therefore, to secure a mean for a species, accurate within ± 10 per cent of the actual mean (for vessel diameter, for example), RENDLE and CLARKE (126) have shown that it is necessary to measure 25 vessels selected at random from each of 5 samples taken from each of 4 trees. Of course such extensive measurements are prohibitory in an investigation of this character. The figures here are not meant to represent anything but very rough estimates of the cell size in the various families. Alone they mean nothing, but where they correlate with all the morphological data it would appear that they have some significance. Under the circumstances, to record the standard deviation and standard error would be most misleading. DESCH (53) has said, "It is important to bear in mind that data collected by bad sampling will become no more illuminating by the calculation of standard errors; such treatment may merely lend spurious support to false conclusions."

The fibrous tracheary elements were not measured, for it was found that these cells could not be measured very accurately in sections; that is, macerated material gave much higher values.

Anatomical descriptions of the families

MORACEAE³

The Moraceae are largely a tropical family with but few representatives in temperate regions. The family is largely arboreal or

³ Anatomical descriptions of this and of the other families studied are given by SOLEREDER (136), RECORD and MELL (124), MOLL and JANSSONIUS (110), and DADSWELL and RECORD (52). The anatomy of various genera and species of the Moraceae and of the other families is described by RECORD (119, 122), KANEHIRA (95, 96), TANG (138, 139), BROWN and PANSHIN (40), JONES (94), BROWN (39), JEFFREY (87), GARRATT (71), RECORD and GARRATT (123), TUPPER (146), KOEHLER (99), and GAMBLE (70).

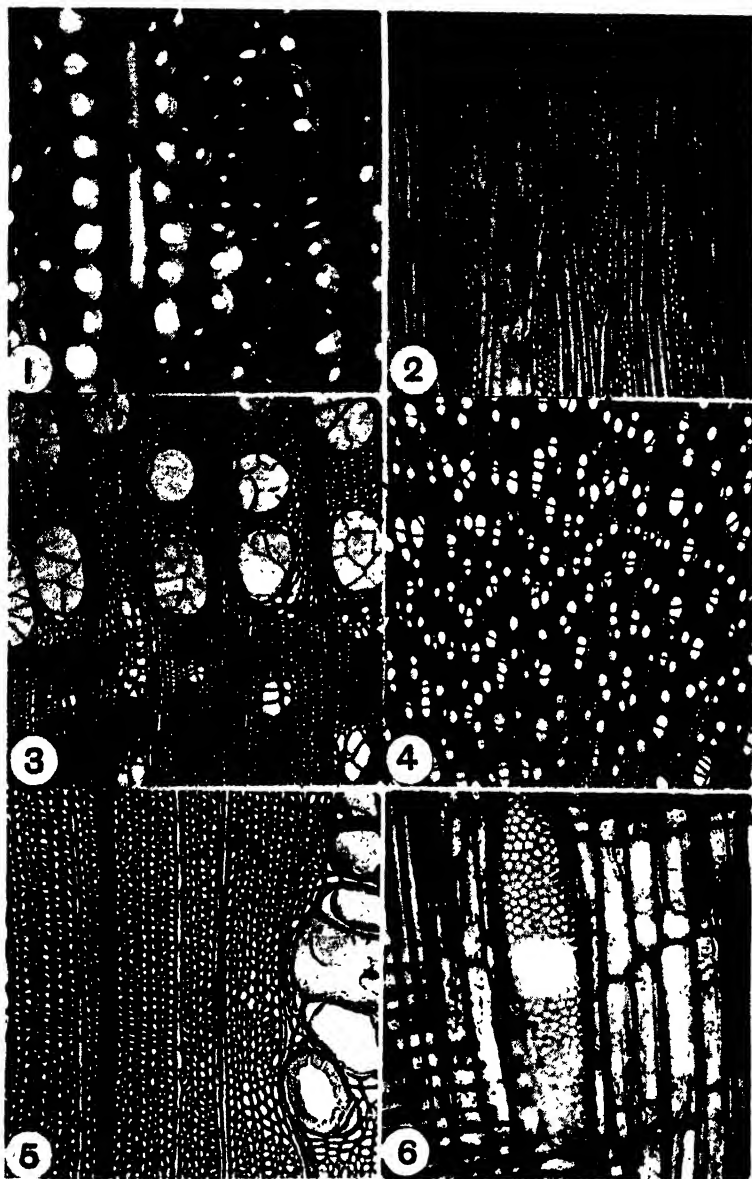
shrubby, with very few herbaceous forms. According to ENGLER and PRANTL (61), there are 73 genera and about 1000 species in the Moraceae. Fifty genera and 100 species were examined in this investigation. The genera studied include: *Trophis* (3 spp.), *Morus* (2 spp.), *Pseudomorus*,⁴ *Paratrophis*, *Pseudostreblus*, *Maclura*, *Chlorophora* (2 spp.), *Bagassa*, *Malaisia*, *Plecosperrum*, *Broussonetia* (2 spp.), *Taxotrophis*, *Streblus*, *Phyllochlamys*, *Sloetia* (2 spp.), *Trymatococcus* (2 spp.), *Craterogyne* (young material), *Helianthostylis*, *Sorocea* (2 spp.), *Clarisia* (2 spp.), *Cudrania* (3 spp.), *Parartocarpus* (2 spp.), *Artocarpus* (3 spp.), *Perebea* (2 spp.), *Helicostylis* (3 spp.), *Castilloa* (4 spp.), *Olmedia* (2 spp.), *Pseudolmedia* (3 spp.), *Antiaris* (3 spp.), *Brosimum* (4 spp.), *Piratinera* (4 spp.), *Bosquiea*, *Ficus* (8 spp.), *Sparattosyce*, *Musanga*, *Myrianthus* (3 spp.), *Coussapoa* (4 spp.), *Pourouma* (3 spp.), *Cecropia* (3 spp.), *Cannabis* (herb), *Humulus* (herb), *Brosimopsis*, *Gymnartocarpus*, *Poulsenia*, *Prainea* (2 spp.), *Anonocarpus*, *Noyera*, *Olmedioperebea*, *Ogcodeia* (3 spp.), and *Mesogyne*.

Most of the species do not have growth rings; in fact, only 18 species were found with them.

Forty-four of the species have fiber-tracheids (fig. 1) predominantly, but the borders on the pits of the fibers are very small. Twelve of these species have septate fiber-tracheids (fig. 2), 8 species with septate fiber-tracheids alone and 4 with but a few septate fiber-tracheids among the non-septate fiber-tracheids. Fifty-four of the species have libriform wood fibers (fig. 56) predominantly. Sixteen of these species have septate wood fibers; 11 with septate wood fibers alone and 5 with but a few septate wood fibers.

Vessel distribution is mostly a combination of solitary pores and pore multiples, with the percentage of solitary pores high compared with the percentage of pore multiples. Some forms have pore clusters (fig. 3) and very few have pore chains, in addition to the other types. Most of the species are diffuse-porous (fig. 4); only 7 species are ring-porous (fig. 3). The vessels are usually (79 spp.) round (fig. 3) in cross section; some (13 spp.) are angular and a few (7 spp.) are round to angular. Most of the species (74) have thin-walled vessels;

⁴ Only one species of each genus was studied in this family and in the other families unless otherwise indicated.

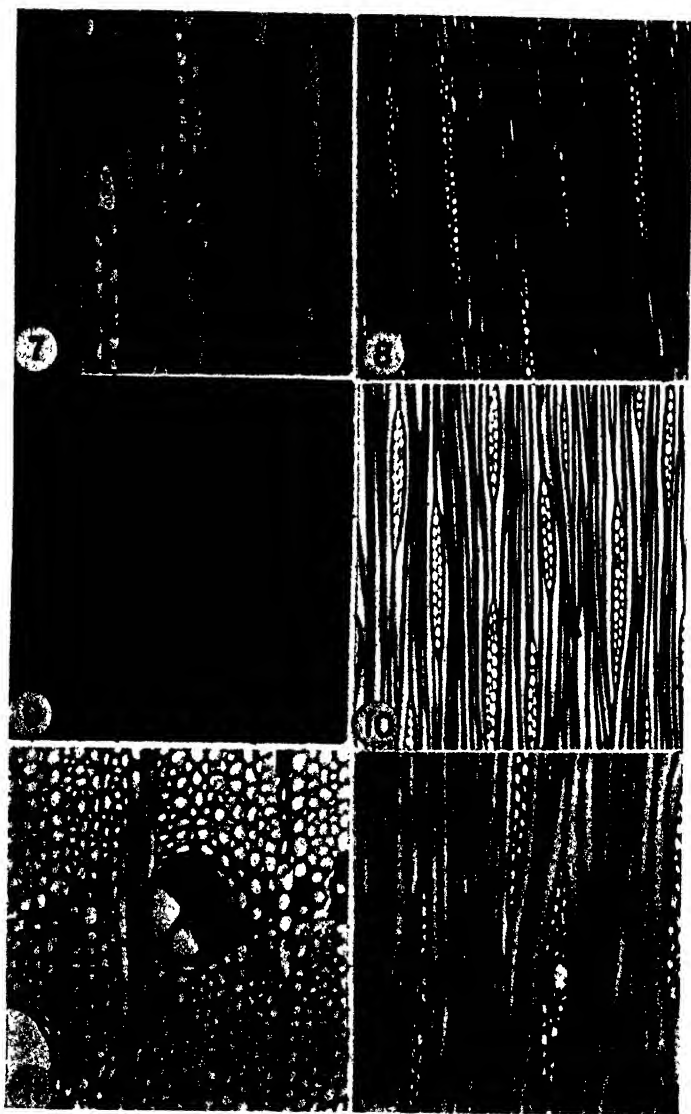


FIGS. 1-6.—Moraceae: Fig. 1, *Parartocarpus venenosa* Becc.; transverse section. $\times 245$. Fig. 2, *Antiaris toxicaria* Lesch.; tangential section. $\times 40$. Fig. 3, *Morus rubra* L.; transverse section. $\times 45$. Fig. 4, *Cannabis sativa* L.; transverse section. $\times 25$. Fig. 5, *Trymatococcus turbinatus* Ducke.; transverse section showing latex tube in a ray. $\times 100$. Fig. 6, *Trophis macrosiachya* Donn.; radial section. $\times 150$.

about a fourth (25 spp.) have vessels with thick walls. The vessel diameters are very small (fig. 4) to large (fig. 3) (averages range 40 to 332 μ ; mean for the family 127 μ). Tyloses are present in most (77) species. These may be few in number (20 spp.) or many (57 spp.) (fig. 3). Most (68) of the species with tyloses have thin-walled tyloses (fig. 3); some (9 spp.) have sclerotic tyloses (fig. 5) with rami-form pits. All the vessel elements have simple perforation plates (fig. 6). The angle which the end walls of the vessel members make with the lateral walls varies from 20° to 90°. Only 13 species have end walls at 90° and then only a few of the end walls are 90°. The intervacular pitting in all is alternate and crowded (fig. 6). The vessel elements are extremely short to medium sized (averages range 125 to 501 μ ; mean of the family 335 μ). There are spiral thickenings in some, or in all of the vessel elements of 9 species—all the forms with ring-porous woods and two other species.

As for ray type, 13 species have heterogeneous I rays, 26 have heterogeneous IIA rays (fig. 7), 53 have heterogeneous IIB rays (fig. 8), 1 has heterogeneous III rays (young material however) (fig. 9), and 6 have homogeneous I rays (figs. 10, 2, 12, 13). In width, the rays vary from 1 to 15 cells. In depth, the uniseriate rays vary from 1 to 57 cells high; the multiseriate rays from 2 to 192 cells high. A number of species (18) have rays which contain a few sheath cells. Ten species have rays with some sclerotic ray cells. Often the forms with sclerotic tyloses have thick-walled fibers, thick-walled vessels, sclerotic ray cells, and sclerotic xylem parenchyma cells (fig. 11). Table 1 shows this correlation.

There are latex tubes in 38 species (fig. 7). These tubes are not intercellular canals but are long laticiferous cells with very obvious cell walls. The latex tubes in the rays are continuous with those in the pith and phloem (fig. 14). Figure 5 shows a latex tube in a transverse section of xylem. The lack of cross walls in the tube is apparent. Similar laticiferous cells have been described in the Apocynaceae (160). The latex tubes in the Moraceae may vary from small (fig. 2) to large (fig. 12). There may be few (10 spp.) or many (28 spp.) in the xylem. They are usually in the center of the ray (fig. 13), rarely near the top; only in one species, *Craterogyne kameruniana* (Engl.) Lanj. (the form with only uniseriate, or hetero-



FIGS. 7-12.—Moraceae: Fig. 7, *Trymalococcus amazonicus* Poepp. and Endl.; tangential section showing latex tube. $\times 200$. Fig. 8, *Sloetia sideroxylon* Teijsm. and Binn.; tangential section. $\times 75$. Fig. 9, *Craterogyne kameruniana* (Engl.) Lanj.; tangential section. $\times 100$. Fig. 10, *Gymmartocarpus woodii* Merrill; tangential section. $\times 50$. Fig. 11, *Helicostylis tomentosa* Rusby; transverse section showing sclerotic xylem parenchyma cells, sclerotic ray cells, and sclerotic tyloses. $\times 120$. Fig. 12, *Parartocarpus venenosa* Becc.; tangential section showing latex tube. $\times 85$.

geneous III rays), are some of the latex tubes outside of the rays. No latex tubes were observed in the sub-families Conocephaloideae and Cannaboideae.

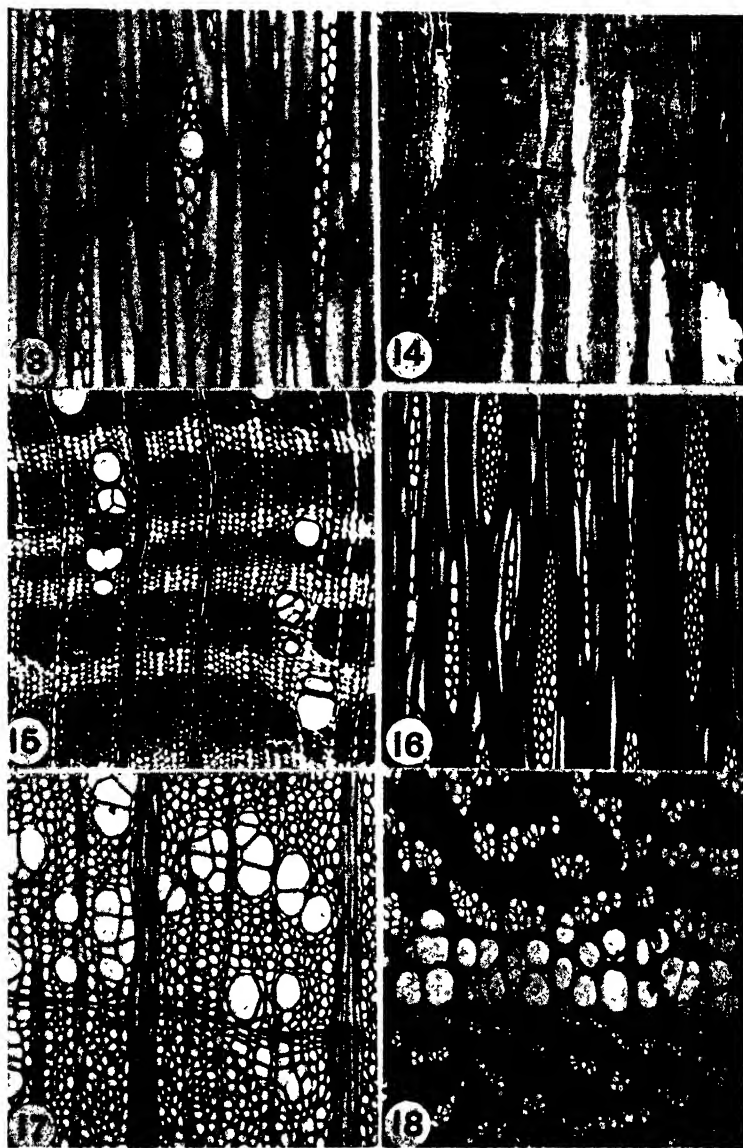
The xylem parenchyma distribution is mostly a combination of aliform and confluent (32 spp.) or metatracheal (24 spp.) (fig. 15).

TABLE 1

CORRELATION BETWEEN PRESENCE OF SCLEROTIC TYLOSES AND
PRESENCE OF OTHER THICK-WALLED ELEMENTS

NAME OF PLANT	SCLEROTIC TYLOSES	FIBER WALL	VESSEL WALL	SCLEROTIC RAY CELLS	SCLEROTIC WOOD PARENCHYMA CELLS
<i>Trymatococcus turbatus</i> (Baill.) Ducke....	+	Fairly thin	Thin	—	—
<i>Cudrania spinosa</i> Tréc....	+	Very thick (hardly any lumen)	Thick	—	—
<i>Cudrania javanensis</i> Tréc.....	+	Very thick (hardly any lumen)	Thick	+	+
				(few)	(many)
<i>Helicostylis tomentosa</i> (P. & F.) Rusby.....	+	Fairly thick	Thin	+	+
				(quite a few)	(many)
<i>Pseudolmedia spuria</i> (Sw.) Gris.....	+	Very thick (hardly any lumen)	Fairly thick	+	+
<i>Piratinera guianensis</i> Aubl.....	+	Thick	Fairly thin	+	+
				(many)	(few)
<i>Piratinera scabridula</i> Blake.....	+	Very thick (hardly any lumen)	Fairly thin	+	+
		Thick	Thick	(many)	(few)
<i>Piratinera velutina</i> Blake	+			+	+
				(many)	(all)
<i>Piratinera panamensis</i> Pittier.....	+	Thick	Thick	+	—

In the latter type the bands may vary from 1 to 22 cells in width. In other species there are various combinations of vasicentric, aliform, confluent, and metatracheal. Only 4 species have terminal parenchyma. None of the species examined shows either diffuse or the absence of xylem parenchyma. *MacLura aurantiaca* Nutt. is the only form with fusiform wood parenchyma cells. Seven species have sclerotic xylem parenchyma cells (fig. 11).



FIGS. 13-18.—Fig. 13, *Parartocarpus triandrus* J. J. Smith, Moraceae; tangential section showing latex tube in a ray. $\times 90$. Fig. 14, *Ficus populoides* Warb., Moraceae; radial section showing latex tube in pith (left) bending out through xylem (center) to phloem (right). $\times 25$. Fig. 15, *Malaisia scandens* Planch., Moraceae; transverse section. $\times 55$. Fig. 16, *Ficus benghalensis* L., Moraceae; tangential section. $\times 65$. Fig. 17, *Planera aquatica* J. F. Gmel., Ulmaceae; transverse section. $\times 80$. Fig. 18, *Ulmus americana* L., Ulmaceae; transverse section. $\times 25$.

There is a tendency toward storied structure in *M. aurantiaca* and *Ficus benghalensis* L. (fig. 16).

The vessel-parenchyma pitting is largely (90 spp.) alternate. Only 6 species have both opposite and alternate pitting. Seventy-six species have some unilateral compound pitting.

Very often there are crystals in the ray cells, or in the wood parenchyma cells, or both. Frequently there are chains of chambered parenchyma cells bearing crystals among the wood parenchyma cells, or among the upright cells of the rays, or both.

Two herbs, *Cannabis sativa* L. (fig. 4) and *Humulus japonicus* Sieb. and Zucc., were examined. Internodal transverse sections reveal the fact that both of these plants are of the "continuous" herb type (that is, the secondary wood forms a complete ring and is not broken up into a number of discrete bundles as in *Aristolochia* or *Clematis*). At the nodes the solid rings are slightly broken up in places, owing to the fact that the leaf traces are flanked by thin layers of parenchyma.

In general it may be said that anatomical specialization has proceeded from the Moroideae to the Artocarpoideae to the Conocephaloideae to the Cannaboideae. However, there are two complicating factors in the anatomical evolution of this family. First, although most of the members of the Moroideae are primitive as far as most anatomical characters are concerned, certain temperate members are rather highly specialized in a number of vessel characters. About a third of the species examined in the Moroideae have some vessel elements with end walls at 90° , a much higher percentage than was found in the other sub-families, excluding the Cannaboideae. Further, in the Moroideae there are many more woods with thick-walled vessels and with ring-porosity than in the other sub-families. Also vessel element length is less than this length in all the sub-families except the Cannaboideae. Thus, in the sub-family Moroideae there are many anatomically primitive woods as well as some of the most highly specialized woods in the family. The explanation for this situation seems to lie in the fact that all except one of the temperate, ring-porous woods of the family are in the Moroideae. An examination of these ring-porous woods, as well as those of other families, reveals that many other characters

in these woods are specialized, even though the nearest relatives are quite primitive anatomically. Table 2 contains a summary of the chief characters of the ring-porous woods of the Moraceae. All the species listed, with the exception of *Cudrania pubescens* Tréc., are in the Moroideae. It can be seen from this table that in these ring-porous species the vessel elements are exceedingly short to very short, the vessels are round in transverse section, some vessel mem-

TABLE 2
DATA ON RING-POROUS WOODS OF THE MORACEAE

NAME OF PLANT	VESSEL ELEMENT LENGTH (μ)	SHAPE OF VESSEL IN CROSS SECTION	THICKNESS OF VESSEL WALL	END WALL OF VESSEL ELEMENTS	SPIRAL THICKENINGS IN VESSEL ELEMENTS	RAY TYPE	TYPE OF FIBROUS TRACHE- ARY ELEMENT
<i>Morus alba</i> L. . . .	178	R*	Some fairly thick; most thin	Few with 90°	+	Heterog. IIB	L. w. f.*
<i>Morus rubra</i> L. . .	161	R	Thin	Some with 90°	+	Heterog. IIB	L. w. f.
<i>Maclura aurantiaca</i> Nutt.	154	R and A*	Thick	Some with 90°	+	Heterog. IIB†	L. w. f.
<i>Plecosperrum spi-</i> <i>nosum</i> Tréc. . . .	231	R	Thick	Few with 90°	+	Homog. I	L. w. f.
<i>Broussonetia papy-</i> <i>rifera</i> (L.) Vent.	214	R	Thin	Few with 90°	+	Heterog. IIB	L. w. f.
<i>Broussonetia kazi-</i> <i>noki</i> Sieb.	233	R	Thin	Some with 90°	+	Heterog. IIB	L. w. f.
<i>Cudrania pubescens</i> Tréc.	155	R	Thick	Some with 90°	+	Heterog. IIB†	L. w. f.

* R, round; A, angular; L. w. f., libriform wood fibers.

† Almost homogeneous I.

bers in each species have end walls at 90°, the vessel elements contain spiral thickenings, the rays are of a high type for the family, and all the fibers are of the highest type. This matter will be discussed later in greater detail, but at this point it may be said that it appears as though the advent of ring-porosity, or of some factor (or factors) causing ring-porosity, gives an impetus to the evolution of certain vessel features as well as to the evolution of other xylary structures. Thus on the whole the Moroideae are the most primitive sub-family in the Moraceae, yet the development of ring-porosity in certain species has led to a general speeding-up of evolutionary development in the xylem of these forms, so that they are more

highly specialized than some of the species in the higher sub-families.

The second complication appears in the sub-family Cannaboideae. The fact that all the plants in this group are herbs and the fact that in almost all anatomical characters this sub-family is higher than the other sub-families, would indicate that on anatomical grounds the Cannaboideae are the highest sub-family in the Moraceae. But the Cannaboideae appear to be primitive as far as a few characters are concerned; that is, the vessels in cross section are angular to round, the vessel diameter is smaller than in the other sub-families, and all the rays are of the lowest type—heterogeneous I. This situation clears, however, when it is remembered that these plants are all herbaceous and that the xylem of an herb is essentially the first annual ring of the plant (133, 134). In other words, the xylem in herbs is not homologous to the xylem taken some distance from the pith of trees. The xylem of herbs is essentially “young material” and is subject to the dangers of interpretation reviewed in the introduction of this study. It is not surprising therefore to find in the otherwise highly specialized and herbaceous Cannaboideae, that the vessels are angular to round in cross section, that the vessel diameters are low, and that the rays are heterogeneous I. If these herbs were to live year after year, adding growth layers each year, one might well expect to find round pores with large diameters and rays of a high type in the hypothetical mature wood of these plants.

Before passing to a consideration of a few general conclusions based on a study of the tribes of the Moraceae, the dispersal of septate fibrous tracheary elements in the family should be given brief attention. The sub-family Artocarpoideae are the center of development of septate fibers, for 10 of the 12 species with septate fiber-tracheids and 15 of the 16 species with septate wood fibers belong in this sub-family. There are no septate fibers in the Moroideae if one excludes the genus *Mesogyne*. Likewise there are no septate fibers in the Conocephaloideae if one excepts the genus *Prainea*. There are no septate fibers in the Cannaboideae.

As for the tribes of the Moraceae, the following conclusions, based on the species examined, may be stated: There are no septate fibers

in the Fatoucae, Moreac, Broussonetieae, Strebleae, Euartocarpeae, Brosimeae, and Cannaboideae. The Dorstenieae have no septate fibers in any of the genera with the exception of *Mesogyne* previously mentioned. All the Olmedieae have some septate fibers. The Ficeae have no septate fibers except for one species, *Ficus religiosa* L. The situation with regard to septate fibers in the Conocephaloideae has already been stated. All the Dorstenieae have fiber-tracheids except *Mesogyne*. Most of the Euartocarpeae have fiber-tracheids; one species, *Brosimopsis diandra* Blake, was found with libriform wood fibers. All the Brosimeae, Strebleae, Ficeae, and Cannaboideae have libriform wood fibers. There are no latex tubes in the Fatoucae, Moreac, Strebleae, Conocephaloideae, and Cannaboideae.

It can be seen that the anatomy of the Moraceae is of rather a high type, for the vessel members have simple perforation plates; more than half of the species have libriform wood fibers; the intervascular pitting is alternate; the xylem parenchyma distribution is aliform, confluent, or metatracheal; the vessel members are short and fairly wide; and most of the vessels are round in cross section. On the other hand, the family is predominantly arboreal in habit.

This description of the anatomy of the Moraceae is in substantial agreement with the accounts in the books and papers cited at the beginning of this description, as well as with the work of BOULTON and PRICE (37).

ULMACEAE

The Ulmaceae are distributed in both tropical and temperate regions but mostly in the north temperate zone. There are trees and shrubs in this family but no herbs. According to ENGLER and PRANTL (61) there are 14 genera and about 140 species. Nine genera and 29 species were examined in the present study. The genera investigated are *Phyllostylon*, *Holoptelea*, *Ulmus* (12 spp.), *Planera*, *Celtis* (9 spp.), *Ampelocera*, *Zelkova* (2 spp.), *Trema* (young material), and *Aphananthe*.

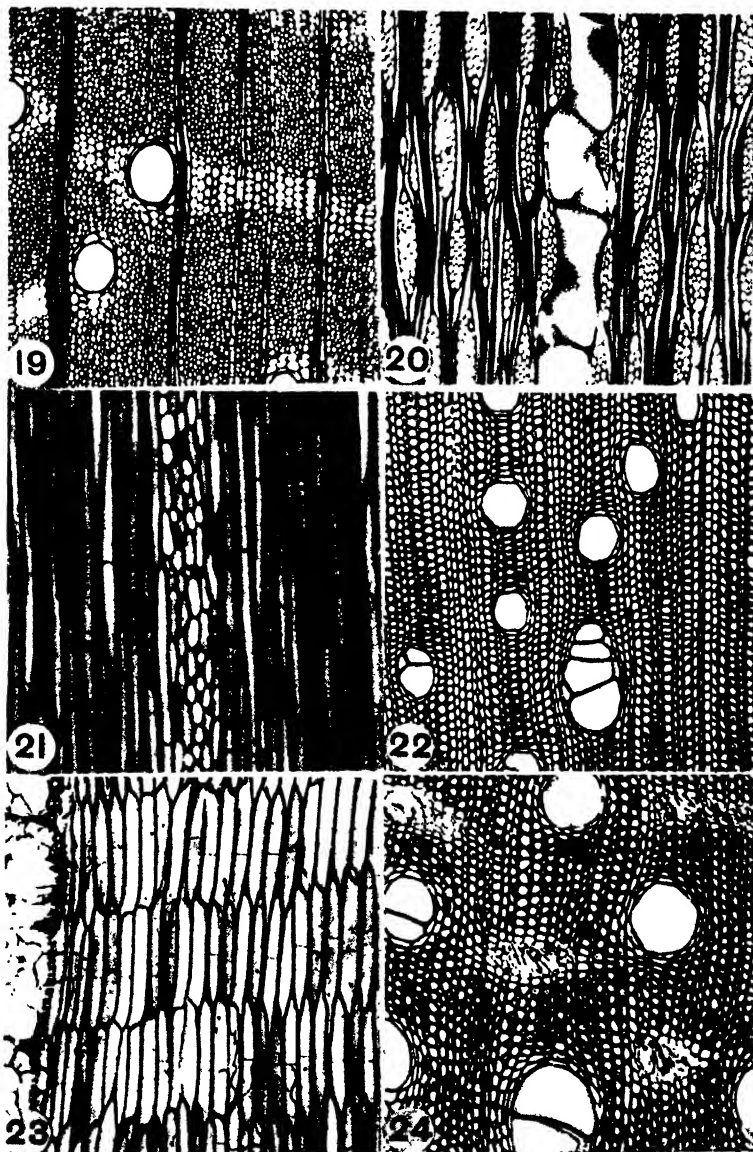
All but two of the species have growth rings (fig. 17).

The fibrous tracheary elements are either fiber-tracheids or libriform wood fibers; if fiber-tracheids, the borders of the pits are small. Four species have fiber-tracheids (fig. 49), 1 species has both fiber-

tracheids and libriform wood fibers, and 24 species have libriform wood fibers. There are no septate fibers of any character in the species examined.

Vessel distribution is a combination of solitary pores, pore multiples, and pore clusters (figs. 17, 18), or a combination of solitary pores and pore multiples. In either case the percentage of solitary pores is always greater than the percentage of pore multiples. Thirteen of the species are diffuse-porous (fig. 17) and 16 species are ring-porous (fig. 18). Two species have angular (fig. 17) vessel outlines in cross section, 9 have angular and round (fig. 19), and 10 have round. Most of the species (15) have thin vessel walls (fig. 17), others (2 spp.) have thin and thick walls, and a few (4 spp.) have thick walls. The vessel diameters are very small (fig. 17) to rather large (fig. 18) (averages range 37 to 166 μ ; mean of the family 96 μ). Tyloses are present in only a few (6) species (fig. 18). In these species the tyloses are many and thin-walled. Ten of the species have vessel elements with simple perforation plates and with some of the vessel elements bearing vestigial scalariform perforation plates (fig. 50). Twelve species have vessel elements with exclusively simple perforation plates. The angle which the end walls of the vessel members make with the lateral walls (fig. 20) varies from 20° to 90°. Eleven species have a few vessel elements with end walls at 90°; 10 species have no end walls at 90°. The intervacular pitting is opposite and alternate in 3 species and alternate (fig. 20) in 18 species. In both groups of species the pitting is crowded. The vessel elements are extremely short to medium sized (averages range 185 to 451 μ ; mean of the family 286 μ). Most of the species (21) have spiral thickenings in some or in all of the vessel elements. As in the Moraceae, all the ring-porous species (16) have spiral thickenings. In addition, 3 species with a tendency toward ring-porosity and 2 species with diffuse-porous wood have spiral thickenings.

As for ray type, 7 species have heterogeneous I rays, 4 species have heterogeneous IIA rays, 5 species have heterogeneous IIB rays (fig. 20), and 13 species have homogeneous I rays. The ray width varies from 1 to 20 cells. In depth, the uniseriate rays run from 1 to 27 cells high and the multiseriate rays from 4 to 160 cells high. A few forms (5 spp.) have sheath cells in the rays.



FIGS. 19-24. - Fig. 19, *Aphananthe aspera* Planch., Ulmaceae; transverse section. $\times 55$. Fig. 20, *Holoptelea integrifolia* Planch., Ulmaceae; tangential section showing storied arrangement. $\times 60$. Fig. 21, *Myriocarpa magnifica* Rusby, Urticaceae; tangential section. $\times 90$. Fig. 22, *Boehmeria macrophylla* Hornem., Urticaceae; transverse section. $\times 70$. Fig. 23, *Urera baccifera* Gaudich, Urticaceae; tangential section showing storied arrangement. $\times 70$. Fig. 24, *Gyrolaenia myriocarpa* Griseb., Urticaceae; transverse section showing island type of unlignified areas of xylem parenchyma. $\times 70$.

Usually the xylem parenchyma distribution is either a combination of terminal and vasicentric arrangements (6 spp.) (fig. 18), or is aliform and confluent (4 spp.) (fig. 19). In other species the parenchyma distribution may be various other combinations of the preceding types and of the metatracheal arrangement. In only 1 species, *Planera aquatica* (Walt.) J. F. Gmel. (fig. 17), is the parenchyma diffuse and vasicentric.

There is a tendency toward storied structure in 3 species and well developed seriation of rays and vessel elements in 2 species (fig. 20).

The vessel-parenchyma pitting is opposite and alternate in 3 species, and alternate in 25 species. Only 1 species has unilateral compound pitting.

Very often there are crystals in the ray cells, or in the wood parenchyma cells, or in both. One species has crystalliferous chambered parenchyma cells among the xylem parenchyma strands.

On the whole the Ulmaceae seem more primitive anatomically than the Moraceae. In the former there are no herbs and no septate fibers. Half of the species have vessels with angular, or angular and round outlines in cross section. In general the vessel diameters are smaller than in the Moraceae. There is some opposite and alternate intervacular pitting in the Ulmaceae, whereas it is invariably alternate in the Moraceae. Lastly and most important of all, in the Ulmaceae some of the species have vestigial scalariform perforation plates in addition to the simple type. This would indicate that the Ulmaceae are more closely allied than are the Moraceae to some group with scalariform perforation plates on the vessel elements. The work of BAILEY and TUPPER (18) and FROST (64, 65) indicates that the type of perforation plate on the vessel members is a very strong phyletic character. There is no evidence that this character is readily modified by changes in the environment.

On the other hand, the Ulmaceae seem to be higher than the Moraceae as far as certain vessel characters are concerned. There are many more ring-porous woods in the Ulmaceae. Half of the species have some vessel elements with the end walls at 90°. The length of the vessel members is less in the Ulmaceae. More than half of the species have spiral thickenings. Most of the woods have libriform wood fibers. Over a third of the species have homogeneous

I rays. There is slightly more storied structure in the Ulmaceae than in the Moraceae.

The key to this situation seems to be the comparatively large number of ring-porous forms in the Ulmaceae. It will be remembered that in the Moraceae, the species with ring-porous woods were much higher anatomically than their immediate diffuse-porous relatives. It appears that the advent of ring-porosity, or some factor (or factors) causing ring-porosity, gives impetus to anatomical specialization. So the Ulmaceae appear to be a group which is more primitive

TABLE 3
DATA ON RING-POROUS WOODS OF THE ULMACEAE

NAME OF PLANT	VES- TIGIAL PER- FORA- TION PLATES	VESSEL ELE- MENT LENGTH (μ)	SHAPE OF VESSEL IN CROSS SECTION	THICK- NESS OF VESSEL WALL	END WALL OF VESSEL ELEMENTS	SPIRAL THICK- ENINGS IN VES- SEL ELE- MENTS	RAY TYPE	TYPE OF FIBROUS TRACHEARY ELEMENT
<i>Ulmus americana</i> L.	+	237	R*	Thin	Few with 90°	+	Homog. I	L.w.f.*
<i>Ulmus mexicana</i> Planch.f.	+	243	R	Thin	None with 90°	+	Homog. I	L.w.f.
<i>Celtis occidentalis</i> L.	+	298	A* and R	Thin	Few with 90°	+	Heterog. IIB	L.w.f. and F.t.* (some)
<i>Celtis sinensis</i> Pers.	+	300	R and A	Thin	Few with 90°	+	Heterog. I†	L.w.f.
<i>Zelkova serrata</i> Makino.	—	185	R and A	Thin	None with 90°	+	Heterog. IIA	F.t.

* R, round; A, angular; F.t., fiber-tracheids; L.w.f., libriform wood fibers.

† Tendency toward ring-porosity.

‡ Almost heterogeneous IIA.

than the Moraceae in many ways; yet seemingly because this family is largely in the temperate region and has developed ring-porosity, the evolution of many other characters has been speeded up.

It is significant that when the ring-porous forms of the Ulmaceae are compared with those of the Moraceae, the latter are higher anatomically than are the former (*cf.* tables 2 and 3). Likewise when the diffuse-porous woods of the two families are compared, the woods of the Ulmaceae are found to be more primitive. In other words, the fact that the proportion of ring-porous to diffuse-porous woods in the Ulmaceae is higher than in the Moraceae seems to explain why the mean vessel element length is shorter and why there are a

larger number of forms with vessel element end walls at 90° , with libriform fibers, with homogeneous I rays, and with storied structure in the Ulmaceae than in the Moraceae.

The description of the anatomy of this family is in substantial agreement with the descriptions in the books and papers cited on page 13, as well as with the work of JANSSONIUS (86).

URTICACEAE

The Urticaceae are widely distributed in both temperate and tropical zones, but they are most abundantly distributed in the tropics. The family is made up largely of herbs, some shrubs, and (very rarely) trees. According to ENGLER and PRANTL (61) there are 41 genera and about 550 species in this family. Twelve genera and 30 species were examined in the present study. The genera investigated are *Urtica* (perennial or annual herbs) (2 spp.), *Gyrotaenia* (shrubs or trees) (fairly young material), *Urera* (shrubs or trees) (3 spp.) (fairly young); *Laportea* (perennial herbs, shrubs, or trees) (4 spp.) (fairly young), *Boehmeria* (small trees, shrubs, undershrubs or herbs) (5 spp.) (fairly young), *Neraudia* (shrubs) (2 spp.) (young), *Pipturus* (trees and shrubs) (3 spp.) (fairly young), *Touchardia* (shrubs) (young), *Debregeasia* (shrubs), *Villebrunea* (shrubs) (3 spp.), *Leucosyke* (trees or shrubs) (fairly young), and *Myriocarpa* (trees or shrubs) (4 spp.) (fairly young).

Only 3 species have growth rings.

The fibrous tracheary elements are either fiber-tracheids or libriform wood fibers; if fiber-tracheids, the borders of the pits are small. Two species have fiber-tracheids alone (both of these have septate fiber-tracheids, exclusively). Fourteen species have both fiber-tracheids and libriform wood fibers: 3 of these have non-septate fibrous elements; 4 have some septate fiber-tracheids and septate wood fibers along with the non-septate fiber-tracheids and libriform wood fibers; and 7 have all septate fiber-tracheids and septate wood fibers (fig. 21). Six species have libriform fibers: 4 of these have the non-septate type and 2 have the septate type. To summarize the fiber situation in another way: 7 species have non-septate fibers and 15 have septate fibers.

Vessel distribution is a combination of solitary pores and pore

multiples (fig. 22). In only 1 species are pore clusters found along with these other 2 types of vessel pattern. In any case the percentage of solitary pores is usually higher than the percentage of multiples. All the species are diffuse-porous. As for the vessel outline in transverse section, 1 species is angular, 10 species are angular and round (fig. 22), and 11 species are round. Most of the species (14) have thin vessel walls (fig. 22), 4 have thin and thick, and 4 have thick walls. The vessel diameters are very small to large (averages range 54 to 226 μ ; mean of the family 125 μ). Tyloses are present in 18 species; 15 species with many and 3 with a few tyloses. All those with tyloses have the thin-walled type. All the vessel elements have simple perforation plates. The end walls of the vessels vary from 20° to 90°. Sixteen species have end walls at 90° (fig. 23); 9 of these have a few vessel members with end walls at 90° and 7 have many vessel members with end walls at 90°. Six species do not have any vessel elements with end walls at 90°. All the species have crowded, alternate intervascular pitting. The vessel elements are extremely short to medium sized (averages range 190 to 388 μ ; mean of the family 284 μ). There are no spiral thickenings in the vessels.

As for ray type, 20 species have heterogeneous I rays and 4 species have heterogeneous IIA rays. Only 1 of these species with IIA rays has IIA rays in the strict sense. The others are nearly heterogeneous I rays for they have some upright cells in the multi-seriate portion. The heterogeneous I rays are very odd (fig. 21) for they have upright cells in the multiseriate portion of the ray and yet the uniseriate wings are very short. Furthermore, there are but very few uniseriate rays and these are low; in fact, 2 species have no uniseriate rays. In width, the rays vary from 1 to 18 cells. However, all but 3 species are 1-9 cells wide. In depth, the uniseriate rays vary from 1 to 16 cells; the multiseriate rays from 4 to 350 cells.

Most often (16 spp.) the xylem parenchyma is vasicentric (fig. 22). At times (4 spp.) it is vasicentric and metatracheal; also vasicentric and aliform (1 sp.), or terminal, vasicentric, and confluent (1 sp.). There are some fusiform parenchyma cells in 9 species.

There is storied structure in 9 species (fig. 23). In 6 of these forms the seriation is marked; that is, xylem parenchyma strands, fusiform parenchyma cells, fibers, and vessel elements are storied—but not

the rays. In 3 of the species the seriation is not so marked, being present only in the xylem parenchyma cells. All the species have alternate vessel-parenchyma pitting. All forms have some unilateral compound pitting.

There are crystals in the ray cells of some (5) species. Two species have crystalliferous chambered parenchyma cells among the parenchyma strands.

Fourteen species in 5 genera (*Gyrotaenia*, *Urera*, *Laportea*, *Touchardia*, and *Myriocarpa*) have unignified parenchyma patches in the xylem. These unignified areas occurred in all the species of the above genera examined and 1 species of *Urtica*. They are made up of unignified wood parenchyma strands, unignified fusiform parenchyma cells, and unignified ray cells. The areas may be in the form of islands (fig. 24), or of bands, or of combinations of both. They have no relation to the pores of the wood. In some species the wood parenchyma cells in the unignified zones are very large. At first glance these unignified regions resemble patches of included phloem, but closer examination fails to reveal any evidence of sieve plates on the cells.

The herbaceous members are of the "continuous" stele type. Internodal transverse sections demonstrate that the xylem is in the form of an uninterrupted ring. At the nodes the xylem ring is discontinuous in places, owing to the fact that the traces to the leaves are flanked, and at times subtended by parenchyma. It is interesting to note that some of the herbs have areas of unignified parenchyma cells, as do the shrubs and trees.

On the whole the Urticaceae are higher anatomically than are the Moraceae. Both families have alternate intervacular pitting and simple perforation plates on the vessel members. The Urticaceae are higher, however, in that: they have a higher percentage of forms with septate fibers, they have more storied structure, they are largely herbaceous, the vessel elements are shorter, they have a higher percentage of forms with the end walls of vessel elements at 90°, and all the species have unilateral compound pits. Further, in the Urticaceae are forms with unignified parenchyma areas. BAILEY (11) has indicated that such anomalous structures are indicative of highly specialized woods.

On the other hand, it would appear that the Urticaceae are more

primitive than the Moraceae, for in the Urticaceae there are many forms with angular, or angular to round, pores, the vessel diameter is slightly smaller, and the ray type is lower.

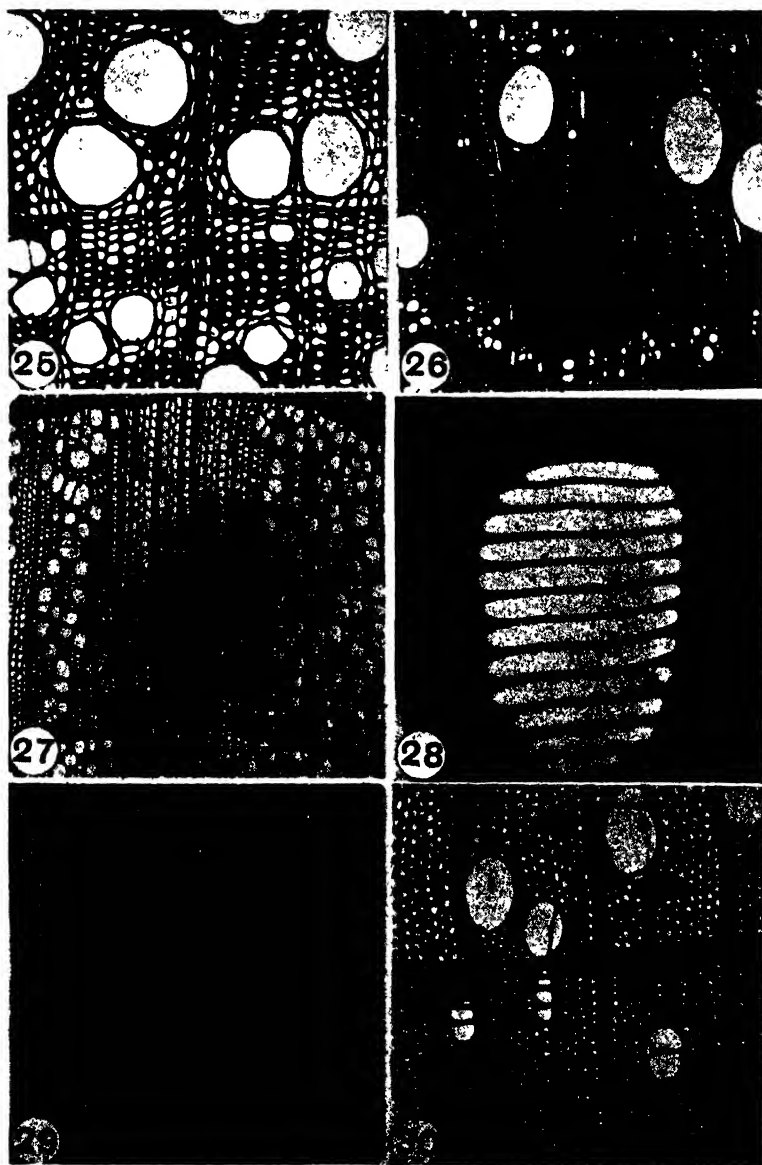
These last few inconsistencies can be explained by the fact that the Urticaceae are mostly shrubs and herbs, and as such they contain but one, or at most a few, growth layers; therefore they consist of "young" wood. With this essentially "young" xylem, one would expect to find that the vessel elements are small, the pores angular, and the rays heterogeneous. The fact that in the forms with heterogeneous I rays, the multiseriate rays have very short wings and that there are very few, or no, uniseriate rays would seem to indicate that these heterogeneous rays might become homogeneous in subsequent growth layers, as happens so often in trees. Further, the species with xylem obviously taken some distance from the pith have heterogeneous IIA rays. It will be recalled that the two highly specialized moraceous herbs, *Cannabis* and *Humulus*, also have heterogeneous I rays. In other words, the Urticaceae occupy the same relation to the Urticales as do the Cannaboideae to the Moraceae.

RHOIPTLEACEAE

The Rhoiptleaceae are a new family (80) which is placed in the Urticales by WETTSTEIN (158) and by ENGLER and DIELS (60). Only one species, *Rhoiptlea chiliantha* Diels and Hand. from Tonking and southwestern China, has been described. It is a tree.

Growth rings are present. All the fibrous tracheary elements are fiber-tracheids. In a transverse section there are many solitary pores, some pore multiples, and a few pore clusters (fig. 25). There is a tendency toward ring-porosity. The vessels are thin-walled and mostly angular to round. The vessels are very small to moderate sized, but mostly small (range 30 to 120 μ ; mean 81 μ). There are many thin-walled tyloses. The perforation plates on the vessel elements are exclusively scalariform. The perforations are non-bordered and wide (4 to 30 μ). There are a few (3 to 8, mostly 3 to 5) bars on each plate. The vessel element end walls vary from 20° to 55°. The intervacular pitting is alternate. These pits are rather small. The vessel elements are medium sized to moderately long (range 640 to 950 μ ; mean 794 μ).

The rays are heterogeneous I, bordering on heterogeneous IIA.



FIGS. 25-30.—Fig. 25, *Rhoiptelea chiliantha* Diels and Hand., Rhoipteleaceae; transverse section. $\times 90$. Fig. 26, *Casuarina equisetifolia* L., Casuarinaceae; transverse section. $\times 80$. Fig. 27, *Alnus japonica* Sieb. and Zucc., Betulaceae; transverse section. $\times 50$. Fig. 28, *Alnus rhombifolia* Nutt., Betulaceae; scalariform perforation plate. $\times 450$. Fig. 29, *Betula grossa* Sieb. and Zucc., Betulaceae; tangential section. $\times 100$. Fig. 30, *Betula davurica* Pall., Betulaceae; transverse section. $\times 90$.

Ray width is from 1 to 5 cells. In depth, the uniseriate rays vary from 2 to 25 cells and the multiseriate rays from 7 to 83 cells.

As for xylem parenchyma distribution, there is a single row of terminal parenchyma, in addition to ordinary vasicentric, aliform, and some confluent (fig. 25). The vessel parenchyma pitting is transitional, opposite, and alternate.

The fact that the perforation plates are exclusively scalariform and that the vessel elements are so long makes it obvious that this family does not belong in the Urticales. The combination of these and other primitive characters, such as the presence of fiber-tracheids, mostly angular vessels, oblique vessel element end walls, heterogeneous I rays, and transitional, opposite, and alternate vessel parenchyma pitting, indicates that this family belongs with some much more primitive order than the Urticales.

The anatomical description given here agrees for the most part with that of TANG (137). However, the writer cannot agree that there is any close resemblance between the ulmaceous genus *Aphananthe* and *Rhoiptelea*. The parenchyma distribution is the same in the two; but then the same combination of vasicentric and terminal parenchyma can be found in dozens of woods. The type of vessel element in the two genera is quite different, as TANG points out.

CASUARINACEAE

The Casuarinaceae are mainly an Australian group, but are also found on the Mascarene Islands, in southeast tropical Asia, on New Caledonia, and on some of the other islands of the Pacific Ocean. They are trees and shrubs. ENGLER and PRANTL (61) state that there are about 30 species in the single genus, *Casuarina*. Eleven species were examined in this study. Six of these species were rather small shrubs, and therefore the wood examined came from a region close to the pith.

There are growth rings in 2 species, traces of growth rings in 4, and none in 5 species.

The fibrous tracheary elements are all tracheids. The bordered pits on the tracheids are not large, but since they are as large as the small bordered pits on the vessel elements, the fibrous elements must be designated tracheids.

Most of the pores are solitary (fig. 26); there are a few pore multi-

ples, however. All the woods are diffuse-porous. The pores are angular in 4 species and angular to round in 7 species. The vessel walls are thin. The vessel diameters are extremely small to moderate sized, but mostly small and moderate sized (range 10 to 140 μ ; mean 88 μ). Seven of the species have scalariform and simple perforation plates on the vessel members; 3 of these have 50 per cent of each class and 4 have mostly simple perforation plates. Three species have vestigial scalariform perforation plates along with the simple. In the 7 species with scalariform and simple perforation plates, all have non-bordered perforations. Six of these species have the intermediate number of bars and 1 species has a few bars. All 7 of these species have narrow perforations. The end walls on the vessel elements vary from 15° to 90°. In 2 species there are some vessel members with end walls at 90°. In all the species the intervascular pitting is transitional (some), opposite (most), and alternate (some). Vessel element length varies from moderately short to medium sized (range 270 to 570 μ ; mean 412 μ). Spiral thickenings are present in some of the vessel members of 4 species.

The rays are heterogeneous IIB, bordering on homogeneous I at times. The rays are from 1 to about 50 cells in width. Aggregate rays are found in all 11 species; absent in old stems in 4 species and present in old stems in 7. Very wide or so-called "compound" rays are present in 6 species. So-called "diffuse" rays are found in 4 species. To summarize the ray situation in another way: in 4 species the aggregate and "compound" rays of the young stems pass over to the "diffuse" condition in the older stems and in 7 species there are aggregate and "compound" rays to both young and old axes. In depth, the uniseriates run from 1 to 18 cells and the ordinary multiseriates run from 5 to 40 cells. The aggregate and "compound" rays are hundreds of cells in height.

The xylem parenchyma is metatracheal in 5 species. Here the bands of parenchyma cells vary from 1 to 7 cells in width. In 3 species there is diffuse and metatracheal parenchyma (fig. 26). The bands are 1 to 4 cells wide. The vessel parenchyma pitting in all is transitional (some), opposite (most), and alternate (some).

Some of the species have chambered parenchyma cells with crystals.

It is apparent that the Casuarinaceae are rather highly specialized anatomically. The vessel elements are fairly short, some of the species have vessel members with end walls at 90° , the rays are almost homogeneous I, the parenchyma is metatracheal, and the intervacular pitting is mostly opposite with some alternate. Furthermore, the vessel elements are mostly simple in 4 species and simple with vestiges of the scalariform state in 3 other species. Anatomically the Casuarinaceae cannot be regarded as the most primitive, or among the most primitive, of the families of the angiosperms, as they have often been considered by certain phylogenists.

On the other hand, the Casuarinaceae are not so highly specialized anatomically as the Urticales. The former have tracheids, angular or angular to round vessels, some scalariform perforation plates, and opposite (mostly) intervacular pitting.

This anatomical description of the Casuarinaceae is in agreement with that of BOODLE and WORSDELL (35).

BETULACEAE

The Betulaceae are characteristic of the north temperate regions of both hemispheres. In the Old World, some species of *Alnus* occur as far south as Bengal, while in the New World 1 species of *Alnus* occurs as far south as Argentina. Species of *Ostrya* and *Carpinus* are found in Mexico. The family consists of trees and shrubs. There are 6 genera and some 80 species in the Betulaceae. The anatomical description which follows is based on a study of 6 genera and 46 species. The genera studied are *Ostryopsis* (shrubs), *Carpinus* (8 spp.), *Ostrya* (3 spp.), *Corylus* (4 spp.), *Betula* (20 spp.), and *Alnus* (10 spp.).

All the species except *Betula cylindrostachya* Wall. have growth rings.

There are some tracheids along with the fiber-tracheids in *Alnus* and *Betula*. In *Carpinus*, *Ostrya*, and *Corylus* some of the fiber-tracheids are quite close to tracheids. *Ostryopsis* has fiber-tracheids.

In 5 genera there are solitary pores, pore multiples, and pore clusters, and in *Ostrya* there are solitary pores and pore multiples. All the genera are diffuse-porous. The vessels are angular in 43

species and angular to round in 3. All the forms have thin-walled vessels. Vessel diameters are extremely small to moderate sized, but mostly very small (fig. 27) and small (averages range 32 to 66 μ , mean 48 μ). Tyloses are present in *Ostrya*. These tyloses are thin-walled and many. The perforation plates are exclusively scalariform in 37 species (fig. 28). In 9 species (most *Carpinus* species and all the species of *Ostrya*) the plates are mostly simple with some vestigial scalariform plates. By far, most of the scalariform plates are non-bordered (fig. 28). In the 5 genera with scalariform perforation plates, 2 have plates with many bars and 3 have plates with the intermediate number of bars (fig. 28). Two genera have narrow perforations and 3 have wide. Vessel element end walls vary from 15° to 50°. Intervascular pitting is alternate in 5 genera and transitional (some), opposite (most), and alternate (some) in *Alnus*. Vessel element length is medium sized to moderately long, mostly medium sized (averages range 421 to 743 μ ; mean of the family 608 μ). There are spiral thickenings in the vessel elements of 11 species; absent in 35. Four of the species with spiral thickenings have scalariform perforation plates exclusively (*Ostryopsis*, 1 species of *Carpinus*, and 2 species of *Corylus*). Seven of the species with spiral thickenings have simple and vestigial scalariform perforation plates (4 species of *Carpinus*, all 3 species of *Ostrya*).

There are heterogeneous IIB rays in *Ostryopsis* and *Corylus*, but even here the rays are almost homogeneous I. The rays are homogeneous I (fig. 29) in 4 genera. In 2 of the latter genera the rays border on heterogeneous IIB. In width, the rays vary from 1 to 4 cells, except in *Alnus rhombifolia* Nutt. where the rays are very wide (up to 25 cells) or so-called "compound." In depth, the uniseriate rays are from 1 to 50 cells high and the multiseriate rays from 3 to 80 cells, except the species with very wide rays, for here the wide rays are many hundreds of cells high. Aggregate rays (fig. 27) are present in 23 species.

The xylem parenchyma is diffuse, terminal (bands 1 to 3 cells in width), and metatracheal (in the form of inconspicuous, meandering, uniseriate rows) in 9 species. Nine species have terminal (1 to 2 cells in width) and metatracheal parenchyma (in the form of inconspicuous, meandering, uniseriate rows). Nine species have diffuse and

terminal (1 to 3 cells in width) (fig. 30). Ten species have diffuse, terminal (1 cell in width), and vasicentric (just a few cells about the vessels, not forming sheaths). One species, *Betula cylindrostachya* Wall., has diffuse and metatracheal (bands 1-3 cells wide) parenchyma. Vessel-parenchyma pitting is opposite and alternate in *Alnus* and alternate in the other 5 genera.

Crystals are found in the ray cells of 3 genera. Pith flecks are present in some (8) species.

Table 4 is a summary of the anatomical data concerning the tribes Betuleae and Coryleae. It is obvious that the Betuleae are more primitive than the Coryleae. As far as three characters are concerned, however, it would seem as though the Betuleae are higher than the Coryleae: Three species of the Betuleae have angular to round vessels. The mean of the vessel diameter averages in the Betuleae is slightly higher than the mean in the Coryleae. This can be explained by the fact that the measurements on *Ostryopsis* and *Corylus* were made on young material. In the Coryleae, 2 species have heterogeneous IIB rays. However, these rays are almost homogeneous I rays; further they also were found in young material.

The Betulaceae are on a lower plane of anatomical specialization than the Casuarinaceae. The Betulaceae have a much greater range of variation than the Casuarinaceae. Some of the genera are as high as *Casuarina*, therefore, whereas most of the genera are considerably lower. The Betulaceae are lower than the Casuarinaceae in the following ways: most of the vessels are angular, the vessel diameter is less, most species have perforation plates which are exclusively scalariform, the vessel length is greater, the parenchyma distribution is diffuse and vasicentric in 50 per cent of the species, and there are no species with vessel element end walls at 90°. On the other hand, the Casuarinaceae seem more primitive in certain ways, for they have tracheids, a lower type of intervacular pitting, and heterogeneous IIB rays.

The anatomical description here given agrees in the main with the accounts by BAILEY (5, 6, 7), BAILEY and SINNOTT (16), HOAR (81), WETMORE (154, 155), and ABBE (1). On the basis of secondary xylem anatomy, ABBE found that *Betula* and *Alnus* are most primi-

tive, *Corylus* and *Ostryopsis* less so, and *Carpinus* and *Ostrya* the least primitive. The results obtained in the present investigation support ABBE's conclusion.

TABLE 4
DATA ON THE TRIBES OF THE BETULACEAE

	BETULEAE (2 GEN., 30 SPP.) NO. OF GENERA OR SPECIES	CORYLEAE (4 GEN., 16 SPP.) NO. OF GENERA OR SPECIES	PERCENTAGE	
			BETULEAE	CORYLEAE
Tracheids (some)	2	0	100	0
Fiber-tracheids (all)	0	4	0	100
Angular vessels	27	16	90	100
Angular to round vessels	3	0	10	0
Range of vessel diameter averages	44-54 μ	32-66 μ		
Mean of vessel diameter averages	49 μ	47 μ		
Exclusively scalariform perforation plates	30	7	100	43 7
Simple and vestiges of scalariform perforation plates	0	9	0	56 3
Many bars on plates	2	0	100	0
Intermediate bars on plates	0	3	0	100
Transitional, opposite, and alternate intervascular pitting	1	0	50	0
Alternate intervascular pitting	1	4	50	100
End walls of vessel elements	15°-35°	15°-50°		
Range of vessel element length averages	717-743 μ	421-651 μ		
Mean of vessel element length averages	730 μ	547 μ		
Spiral thickenings absent	2	5	100	31.3
Spiral thickenings present	0	11	0	68.7
Heterogeneous IIB rays	0	2	0	50
Homogeneous I rays	2	2	100	50
Ray width	1-4*	1-4		
Uniseriate ray depth	1-28	1-50		
Multiseriate ray depth	5-20*	3-80		
Aggregate rays absent	20	3	66 3	18 7
Aggregate rays present	10	13	33.3	81.3
Diffuse and vasicentric parenchyma	19	0	85.5	0
Metatracheal parenchyma	3	16	14.5	100
Opposite and alternate vessel-parenchyma pitting	1	0	50	0
Alternate vessel-parenchyma pitting	1	4	50	100

* Disregarding very wide rays of *Alnus rhombifolia* Nutt.

FAGACEAE

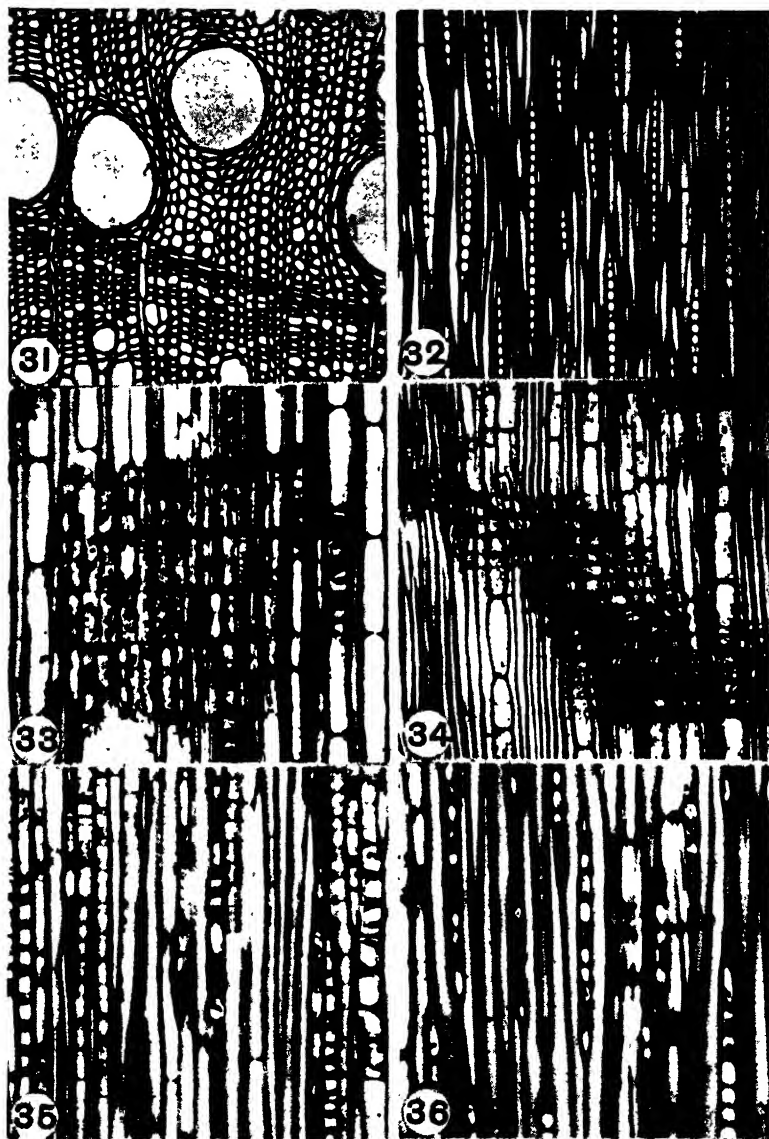
The Fagaceae are found in temperate and tropical regions, but they are absent from tropical and South Africa. The plants are generally trees; there are some shrubs, however. The family consists of

7 genera and about 350 species. The present study is based on an examination of 6 genera and 84 species. The genera include *Fagus* (5 spp.), *Castanea* (4 spp.), *Castanopsis* (4 spp.), *Pasania*, *Pasaniopsis*, and *Quercus* (69 spp.).

All have growth rings except 3 species of *Quercus* (*Q. engelmanni* Greene, *Q. gilva* Blume, and *Q. bennettii* Miq.).

One genus has tracheids and 5 genera have tracheids and fiber-tracheids. The tracheids in the latter group are vasicentric.

The vessels are mostly solitary with some multiples and clusters. Twenty-six species are diffuse-porous and 57 are ring-porous (fig. 31). The vessels are angular in 4 species, angular to round in 15 (fig. 31), and round in 4. The vessel walls are thin in 19 species, thin to thick in 5, and thick in 4. Vessel diameters range all the way from extremely small to very large, but are mostly small to moderate sized (averages range 46 to 120 μ ; mean of the family 75 μ). Tyloses are absent in 1 genus and present in the other 5 genera. The tyloses are thin-walled and many. In one species of oak, *Quercus engelmanni*, the tyloses are sclerotic. The perforation plates on the vessel elements are scalariform and simple in 16 species, of which 2 species have mostly scalariform and 14 have mostly simple. Two species have simple and vestigial scalariform perforation plates. Two species have exclusively simple perforation plates. These perforation plate figures include but 7 species of *Quercus* in order not to distort the picture of the family as a whole by the vessel condition in the many species of *Quercus*. The various species of *Quercus* exhibit considerable variation as regards the type of perforation plate. Some species have simple and scalariform (mostly simple) plates, others have simple and vestigial scalariform, and still others have simple plates exclusively. Most of the forms in the family with scalariform plates have non-bordered apertures; 1 species has the apertures bordered to the middle and 1 species has them bordered at the ends. In 1 species there are many bars, intermediate in number in 2, and a few in 7. The apertures or perforations are wide in all except 1 of the species. The end walls of the vessel members vary from 20° to 90°. *Quercus* has some vessel elements with end walls at 90°; the other 5 genera have no vessel elements with end walls at 90°. The inter-vascular pitting is transitional and opposite in 1 genus, opposite in



FIGS. 31-36.—Fig. 31, *Pasania sieboldii* Makino, Fagaceae; transverse section. $\times 80$. Fig. 32, *Castanea pumila* Mill., Fagaceae; tangential section. $\times 80$. Fig. 33, *Castanopsis argentea* A. DC., Fagaceae; radial section of region close to pith, showing heterogeneous ray. $\times 120$. Fig. 34, same, radial section of region some distance from pith, showing that rays have become quite homogeneous. $\times 100$. Fig. 35, same, tangential section of region close to pith, showing heterogeneous rays. $\times 150$. Fig. 36, same, tangential section of region some distance from pith, showing that rays have become quite homogeneous. $\times 150$.

4, and opposite and alternate in 1. The vessel lengths vary from extremely short to moderately long, but mostly moderately short and medium sized (averages range 290 to 575 μ ; mean of the family 473 μ). It is odd that there are no spiral thickenings in the vessel elements and yet this family has many temperate, ring-porous woods.

The rays are heterogencous IIB in 1 genus, homogeneous I in 1, and homogeneous III (fig. 32) in 4 genera. In 2 of the genera with the latter type of ray, the rays are rarely 2 cells wide. In the other 2 genera the rays are slightly heterogencous in places. Figure 33 is a photomicrograph of a radial section of the xylem of *Castanopsis argentea* A. DC. taken from a region close to the pith. The rays are strikingly heterogeneous in this zone. Figure 34 is a similar view from the same species, but here the xylem pictured is from a region which is a considerable distance from the pith. It is obvious that the rays are homogeneous. In other words, close to the pith the rays are heterogeneous while some distance out they are homogeneous. This heterogeneity of the rays in youthful regions is of great importance in interpreting young material, small shrubs, and herbs. Figure 35 is a tangential view of the rays in a "young" region, and figure 36 is the same in a "mature" region.

The rays vary from 1 to 40 cells in width. In all except *Fagus* the rays are of two distinct types without any intermediate forms; that is, uniseriate rays and very wide, or so-called "compound rays." The uniseriate rays are from 1 to 48 cells high. The multiseriate rays run from 7 cells to thousands of cells high. Very wide rays, or "compound rays," are present in *Fagus*, in *Quercus*, and sometimes in *Castanea*. The other 3 genera have uniseriate rays. *Quercus palmeri* Engelm. and *Q. laevis* Roxb. have no "compound" rays. The former does have aggregate rays; the latter has only uniseriate rays. Aggregate rays are present sometimes in the young wood of *Castanea* and sometimes in the young wood of *Quercus* when they are absent in the mature wood. Seventeen of the 69 species of *Quercus* examined have aggregate rays in the mature wood. The form with the sclerotic tyloses, *Q. engelmanni* Greene, has sclerotic ray cells also.

The xylem parenchyma is diffuse and metatracheal (fig. 31) in

most of the species. The metatracheal parenchyma is in the form of uniseriate rows usually; in 2 species these bands are 1 to 3 cells in width. In 2 species the xylem parenchyma is diffuse. Vessel-parenchyma is transitional and opposite in 1 genus, opposite in 4, and opposite to alternate in 1.

Crystals are found in the ray cells of 2 genera. Crystalliferous chambered parenchyma cells (fig. 35) among the parenchyma strands and among the ray cells are present in some species of *Quercus* and *Castanopsis*.

The Fagaceae are more highly specialized anatomically than are the Betulaceae, for in the Fagaceae the vessels are mostly angular to round, or round; the vessel walls are often thin to thick, or thick; the vessel diameter is greater; the perforation plates of the vessel elements are of a higher type; some species have vessel member end walls at 90°; the vessel element length is less; the ray type is higher; and the woods are often ring-porous. On the other hand, the Betulaceae seem more highly evolved as far as two characters are concerned. The intervacular pitting is mostly alternate, and most of the genera have fiber-tracheids exclusively, whereas in the Fagaceae there are always tracheids present. However, these tracheids in the Fagaceae are vasicentric and therefore highly specialized structures occurring about the large vessels of the ring-porous woods and the vessels of diffuse-porous woods.

The preceding anatomical description of the Fagaceae agrees substantially with the account by BAILEY (5) and with various other descriptions (56, 6, 7, 16, 154, 155) of ray conditions in the family.

JUGLANDACEAE

The Juglandaceae inhabit the warmer parts of the north temperate zone, extending into tropical eastern Asia to China and Japan. The plants of this family are trees placed in 7 genera and some 37 species. The anatomical description given here is based on a study of 5 genera and 18 species. The genera investigated are *Engelhardtia* (2 spp.), *Pterocarya*, *Juglans* (7 spp.), *Carya* (7 spp.), and *Alfaroa*.

Growth rings are present in 16 species; there is a trace in 1 and no growth rings in 1 species.

There are tracheids and fiber-tracheids in 1 species. Seventeen

species have fiber-tracheids exclusively; 6 of the latter have fiber-tracheids which are nearly libriform wood fibers.

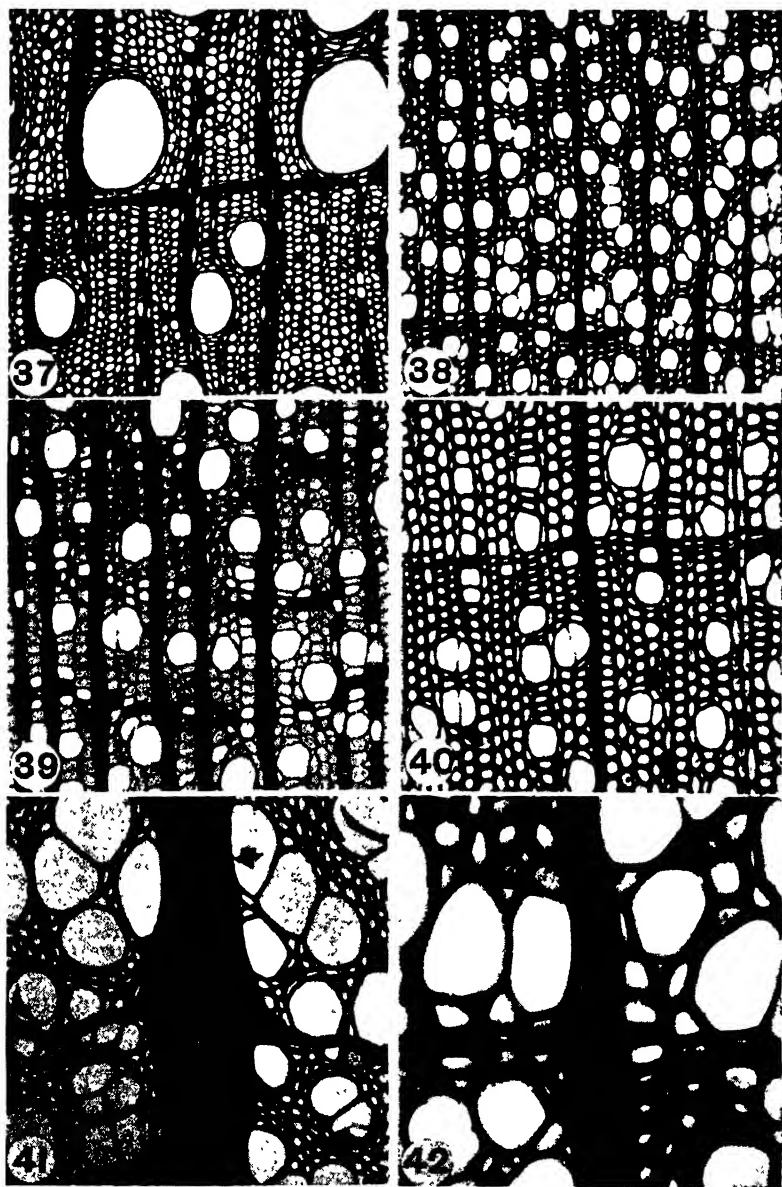
The vessels are solitary, in multiples, and in clusters in 3 genera. In 2 genera the vessels are solitary and in multiples. Eight species are diffuse-porous, 4 are semiring-porous, and 6 are ring-porous (fig. 37). The vessels are angular in 1 species and angular to round in 17 species (fig. 37). The walls of the pores are thin in 11 (fig. 37) and thick in 7 species. The vessel diameters vary from very small to rather large, but mostly small and moderate sized (averages range 68 to 154 μ ; mean of the family 119 μ). Tyloses are present in 16 of the 18 species. In 2 species there are only a few; in 14 species there are many. In any case the tyloses always have thin walls. The perforation plates on the vessel members are scalariform and simple in 2 species, simple and vestigial scalariform in 1, and simple in 15 species. The 2 species with simple and scalariform perforation plates have wide, non-bordered perforations and there are only a few bars to each plate. Vessel element end walls vary from 20° to 75°. Intervascular pitting is transitional (some), opposite, and alternate in 1 species and alternate in 17 species. Vessel member length varies from very short to very long, but is mostly medium sized (averages range 368 to 718 μ ; mean of the family 528 μ).

The rays are heterogeneous I in 1 species, heterogeneous IIA in 4, and heterogeneous IIB in 13 species (2 of the latter species have rays close to IIA). The rays are 1 to 6 cells in width. The uni-seriates are 1 to 40 cells in depth and the multiseriate rays are 4 to 75 cells high.

In most species (7) the xylem parenchyma is terminal and confluent. In 4 species it is terminal, metatracheal, vasicentric, and confluent. In 2 species it is terminal, metatracheal, and vasicentric. In 1 species it is diffuse, metatracheal, and vasicentric and in another it is terminal, metatracheal, and confluent. Vessel-parenchyma pitting is transitional, opposite, and alternate in 1 species and alternate in 17. Unilateral compound pitting is found in 2 species.

Crystals are present in the ray cells of certain species. Crystalliferous chambered parenchyma cells among the wood parenchyma strands are found in 3 species. There are crystals in the swollen xylem parenchyma cells in all the species of *Carya*.

All in all, the Juglandaceae are more highly specialized ana-



FIGS 37-42.—Fig. 37, *Juglans californica* S. Wats., Juglandaceae; transverse section. $\times 55$. Fig. 38, *Hamamelis virginiana* L., Hamamelidaceae; transverse section. $\times 80$. Fig. 39, *Distylium racemosum* Sieb. and Zucc., Hamamelidaceae; transverse section. $\times 90$. Fig. 40, *Stachyurus praecox* Sieb. and Zucc., Stachyuraceae; transverse section. $\times 110$. Fig. 41, *Platanus occidentalis* L., Platanaceae; transverse section. $\times 90$. Fig. 42, *Eucommia ulmoides* Oliver, Eucommiaceae; transverse section. $\times 380$.

tomically than are the Fagaceae. This is evident when it is remembered that in the former the fibrous tracheary elements are of a higher type, the vessels are thick-walled in many species, the vessel diameter is greater, most of the species have simple perforation plates, and most of the species have alternate intervacular pitting. Yet in the Juglandaceae the vessel elements are longer, the ray type is lower, and there are no species with round vessels exclusively. The Juglandaceae are also on a higher plane, anatomically speaking, than are the Casuarinaceae. However, in the Casuarinaceae there are some species with vessel end walls at 90° . Further, the vessel elements are shorter and the ray type higher than in the Juglandaceae.

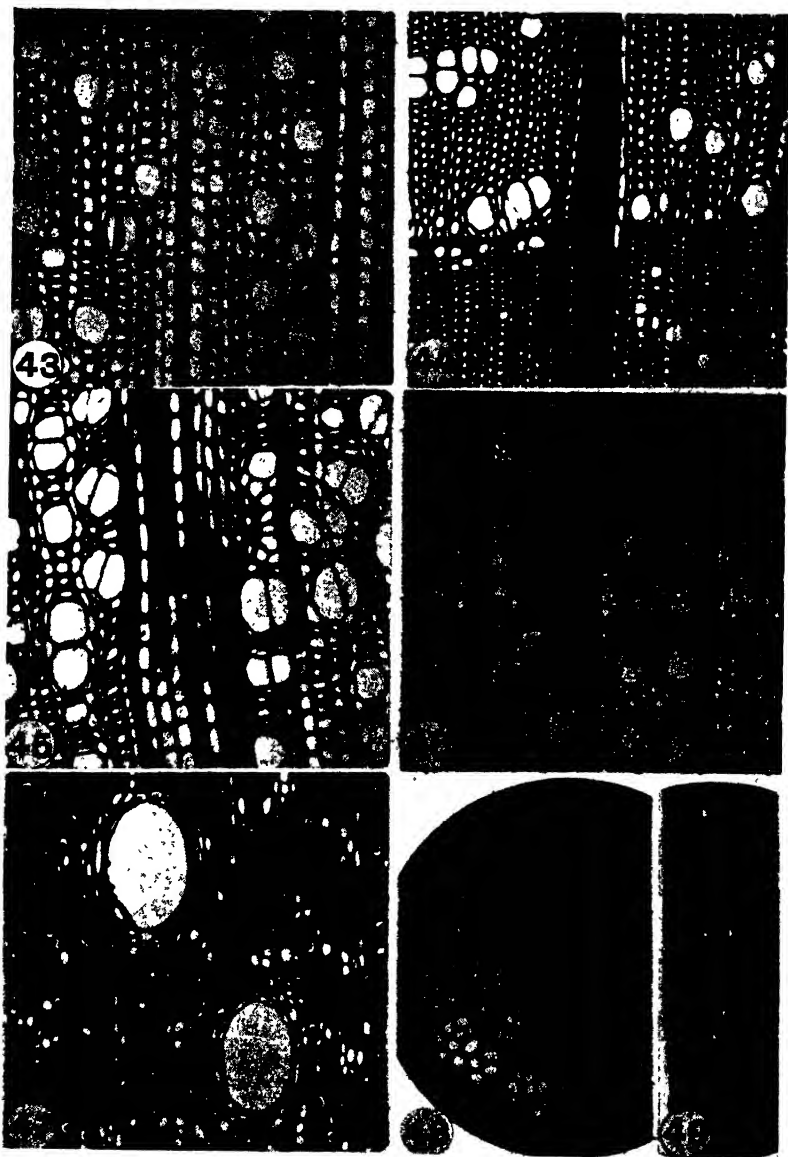
The anatomical description of the family given here is in agreement with that of KRIBS (100).

HAMAMELIDACEAE

The Hamamelidaceae are a family of trees and shrubs occurring in subtropical and temperate climates, especially in Asia and eastern North America. There are a few in Madagascar and South Africa. ENGLER and PRANTI. (62) divide the family into 23 genera and about 94 species. The present investigation is based on a study of 18 genera and 30 species. The genera examined anatomically are *Disanthus* (very young), *Hamamelis* (2 spp.), *Loropetalum*, *Trichocladus*, *Maingaya* (very young), *Eustigma* (very young), *Corylopsis* (4 spp.), *Fortunearia* (very young), *Parrotia*, *Parrotiopsis* (very young), *Fothergilla* (young), *Distylium* (2 spp.), *Sycopsis* (3 spp.), *Sinowilsonia* (very young), *Rhodoleia* (2 spp.) (very young), *Bucklandia*, *Liquidambar* (3 spp.), and *Altingia* (3 spp.).

Growth rings are absent in but 3 species. Vertical intercellular canals, or gum ducts, of the traumatic or gummosis type (as defined by RECORD, 119) are found in 3 species (in *Liquidambar styraciflua* L., but not in the other 2 species; and in *Altingia chinensis* (Oliver) Benth. and *A. obovata* Merr. and Chun., but not in *A. excelsa* Nor.).

The fibrous tracheary elements in 21 species are all tracheids, and tracheids and some fiber-tracheids in 1 species. That these are true tracheids in the BAILEY sense (13) is clear when the pits on the

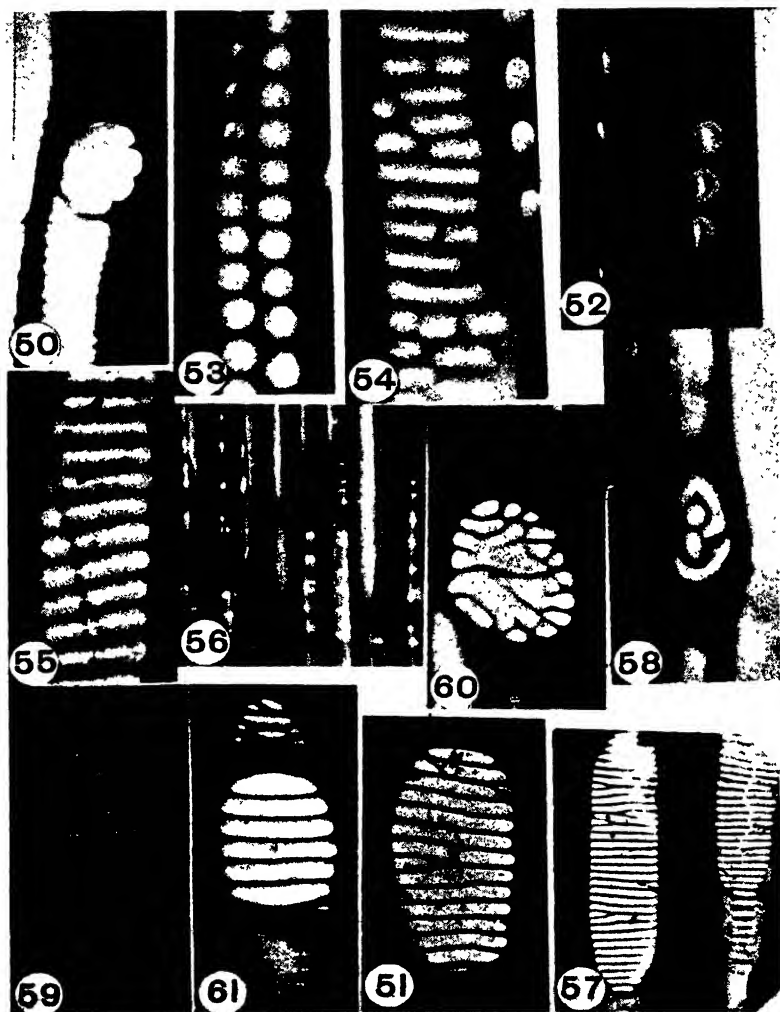


FIGS. 43-49.—Fig. 43, *Hydrangea quercifolia* Bartram, Hydrangeaceae; transverse section. $\times 100$. Fig. 44, *Ribes viscosissimum* Pursh., Grossulariaceae; transverse section. $\times 90$. Fig. 45, *Carpodetus serratus* Forst., Escalloniaceae; transverse section. $\times 90$. Fig. 46, *Pyrus americana* DC., Rosaceae; transverse section. $\times 120$. Fig. 47, *Parinarium gilleii* DeWild., Rosaceae; transverse section. $\times 50$. Fig. 48, *Aristolochia tomentosa* Sims., Aristolochiaceae; transverse section. $\times 13$. Fig. 49, *Planera aquatica* J. F. Gmel., Ulmaceae; fiber-tracheid bearing small bordered pits with extended apertures. $\times 700$.

fibrous elements are compared with those on the vessel members (*cf.* figs. 52, 53).

Vessel distribution in transverse section in all the species is mostly solitary with but a very few multiples (figs. 38, 39). All the species are diffuse-porous (fig. 38). In all, the vessels are angular and thin-walled (figs. 38, 39). Vessel diameters are usually extremely small (fig. 38) and very small, rarely small (averages range 15 to 48 μ ; mean of the family 32 μ). Thin-walled tyloses are found in 4 species. These are few (1 sp.) or many (3 spp.). In all the species the perforation plates on the vessel elements are exclusively scalariform (figs. 51, 57). In 7 species the perforations in the plates are bordered at the ends and in 16 species these apertures are non-bordered (figs. 51, 57). These figures are based on the condition of the majority of the vessels in a species, for in a given species some of the perforation plates, or parts of plates, may vary all the way from completely bordered to non-bordered. The perforation plates have many bars (fig. 57) in 14 species and the intermediate number of bars (fig. 51) in 9 species. The apertures on the plates are narrow (figs. 51, 57) in 19 species and wide in 4. In all the species the vessel end walls are very oblique, varying from 5° to 40°. The intervacular pitting is scalariform (fig. 55), transitional (fig. 54), and opposite (fig. 53) in 18 species. In this group of 18 species the pitting is mostly transitional and opposite, or mostly opposite. The intervacular pitting in 3 species is transitional and opposite; and in 1 other species it is opposite. Vessel element length is medium sized to extremely long, but is mostly moderately long and very long (averages range 760 to 1598 μ ; mean of the family 1089 μ). Spiral thickenings are present in the tips or "ligules" of vessel elements in 9 species, in all parts of the vessel elements in 1 species, and are absent from 20 species.

The rays are heterogeneous I in 9 species, heterogeneous IIA in 8 species, and heterogeneous III in 5 species. In the last group, with uniseriate, heterogeneous III rays, 3 of the species show a tendency to form multiseriate rays and the other 2 species are represented only by young material. Consequently, in older material this group of 5 species with heterogeneous III rays may be found to be heterogeneous I, or IIA. The rays of the family vary from 1 to 5 cells in width. The uniseriate rays are from 1 to 75 cells high and the multiseriates from 3 to 60 cells.



FIGS. 50-61.—Fig. 50, *Ulmus crassifolia* Nutt., Ulmaceae; vestigial scalariform perforation plate and spiral thickenings in vessel members. $\times 480$. Fig. 51, *Hamamelis virginiana* L., Hamamelidaceae; scalariform perforation plate. $\times 350$. Fig. 52, *Liquidambar styraciflua* L., Hamamelidaceae; tracheids with large bordered pits (cf. size of these pits with those on vessel element in fig. 53). $\times 500$. Fig. 53, same, opposite intervascular pitting on vessel element. At the top the pitting appears alternate, owing to a slight twist in the element. $\times 500$. Fig. 54, same, transitional intervascular pitting on vessel element. $\times 500$. Fig. 55, same, scalariform intervascular pitting on vessel element. $\times 500$. Fig. 56, *Trophis racemosa* Urb., Moraceae; simple pits on libriform wood fibers. $\times 510$. Fig. 57, *Corylopsis wilsoni* Hemsl., Hamamelidaceae; scalariform perforation plate. $\times 220$. Fig. 58, *Pyrus americana* DC., Rosaceae; perforation plate with modification of reticulate arrangement. $\times 520$. Fig. 59, *Brunellia comocladifolia* Humb. and Bonpl., Brunelliaceae; transitional intervascular pitting on vessel element. $\times 400$. Fig. 60, *Pyrus americana* DC., Rosaceae; reticulate perforation plate. $\times 450$. Fig. 61, *Ribes viscosissimum* Pursh., Grossulariaceae; scalariform perforation plate. $\times 430$.

Xylem parenchyma is diffuse (fig. 38) in 13 species, diffuse and terminal in 1, diffuse and metatracheal (fig. 39) in 5, and metatracheal in 2. One species has no xylem parenchyma, but this condition may be attributed to the youth of the specimen. Vessel-parenchyma pitting is scalariform, transitional, and opposite in 18 species, transitional and opposite in 3, and opposite in 1.

Crystalliferous chambered parenchyma cells among the wood parenchyma strands are present in 2 species. The same types of cells among the upright cells of the rays are present in 3 different species. In 2 additional species there are crystals in the ray cells.

Anatomically the Hamamelidaceae are the most primitive of all the families considered thus far. All but 1 species have tracheids exclusively; the vessels are mostly solitary; the woods are diffuse-porous; the vessels are angular, thin-walled, and extremely small; the perforation plates are exclusively scalariform; the end walls of the vessel members are very oblique; the intervacular pitting is scalariform, transitional, and opposite; the vessel elements are very long; the rays are usually heterogeneous I, or IIA; and the xylem parenchyma is diffuse in most species.

The secondary xylem of this family is very close in all anatomical details to certain groups in the Magnoliales as described by McLAUGHLIN (109) (the writer has also examined most of the genera of this order). The Magnoliales exhibit a considerable range of anatomical structures, yet it may be said that on the whole the least specialized members of that order are more primitive anatomically than are the Hamamelidaceae. Four genera of the Magnoliales have no vessels (143, 17, 109). Most of the species with scalariform perforations have apertures which are bordered at the ends, or bordered to the middle, or completely bordered. Further, most of the pitting in the Magnoliales is scalariform and transitional. The fibrous tracheary elements are exceptionally long in certain species (18). Some of the species have scalariform pits on the tracheids in the secondary xylem.

STACHYURACEAE

The Stachyuraceae are a small family of shrubs or small trees found in eastern Asia. The family consists of 1 genus and 2 species. The present study is based on a piece of young wood of *Stachyurus praecox* Sieb. and Zucc.

Growth rings are present. All the fibrous tracheary elements are tracheids. Some of the tracheids have spiral thickenings.

Most of the pores are solitary; some are in multiples (fig. 40). The wood is diffuse-porous. The vessels are angular and thin-walled (fig. 40). The pores are extremely small to very small, but mostly very small (range 20 to 42 μ ; mean 34 μ) (fig. 40). The perforation plates on all the vessel elements are scalariform. Most of the perforations are bordered at the ends; some are bordered to the middle. There are many bars (about 36) on the plates and the apertures are narrow. The vessel element end walls are very oblique (7° to 30°). Intervascular pitting is mostly opposite, rarely transitional. The pits on the vessels are small. Vessel element lengths are moderately long to very long, mostly very long (range 1110 to 1300 μ ; average 1225 μ). There are spiral thickenings in the vessel members.

The rays are heterogeneous I and from 1 to 4 cells in width. The uniseriate rays vary from 2 to 33 cells in depth and the multi-seriates from 6 to 65 cells.

The xylem parenchyma distribution is diffuse (fig. 40). Vessel-parenchyma pitting is mostly opposite, rarely transitional.

The anatomy of the Stachyuraceae is very similar to that of the Hamamelidaceae. However, WETTSTEIN (158) and ENGLER and PRANTL (62) place the former in the Parietales in the vicinity of the Flacourtiaceae. It is obvious that mature wood and the xylem of the other species of the Stachyuraceae should be studied before a decision can be made as to which group it is more likely to be related. Judging from the descriptions of the anatomy of the Flacourtiaceae (147, 148), the Stachyuraceae do not belong very close to the former. However, further study may show that the Stachyuraceae are related to the Canellaceae,⁵ or to some other family of the Parietales.

MYROTHAMNACEAE

The Myrothamnaceae are a family of small shrubs occurring in Africa and Madagascar. There is 1 genus and 2 species. A specimen

⁵ VESTAL (148) suggests that the Canellaceae be taken out of the Parietales and placed near the Myristicaceae, as WETTSTEIN and BESSEY have done.

of *Myrothamnus flabellifolia* Welwit., containing about 15 growth rings, was studied in this investigation.

All the fibrous tracheary elements are tracheids. Most of the vessels are solitary; some are in multiples. The wood is diffuse-porous. The vessels are thin-walled and angular. The pores are extremely small to small, mostly very small (range 18 to 52 μ ; mean 35 μ). All the perforation plates are scalariform. There are many bars (about 45) and the perforations are narrow. The vessel member end walls are very oblique (5° to 35°). The intervacular pitting is transitional and opposite. The pits on the vessel elements are very small. Vessel element length is moderately long (range 850 to 960 μ ; mean 905 μ).

The rays are heterogeneous III, and therefore are all uniseriate. In depth, the rays vary from 1 to 25 cells.

No xylem parenchyma was observed. Vessel-parenchyma pitting is transitional and opposite.

Anatomically the Myrothamnaceae resemble the Hamamelidaceae very closely.

BUXACEAE

The Buxaceae are generally shrubs, trees, or rarely herbs. The 7 genera and approximately 30 species have a scattered distribution in temperate and subtropical regions. The present study is largely based on 3 species of *Buxus*, although *Pachysandra terminalis* Sieb. and Zucc. was also examined.

Growth rings are present in 2 species of *Buxus*. The fibrous tracheary elements are all tracheids.

The pores are mostly solitary with a few pore multiples. The wood is diffuse-porous. The vessels are angular and thin-walled. The vessel diameter is extremely small (range 12 to 26 μ ; mean 20 μ). The vessel elements have scalariform and simple perforation plates, but the simple plates are rare. The plates have an intermediate number of bars and are narrow and non-bordered. The end walls of the vessel elements vary from 10° to 50° . Intervacular pitting is mostly opposite, with some alternate. The pits on the vessels are very small. Vessel element length is medium sized to moderately long, mostly medium sized (range 540 to 990 μ ; mean 711 μ).

The rays are heterogeneous IIA and from 1 to 3 cells in width.

The uniseriate rays vary from 1 to 17 cells high and the multiseriate rays from 5 to 25 cells high.

Xylem parenchyma distribution is diffuse. Vessel-parenchyma pitting is opposite and alternate.

ENGLE and DIELS (60) place this family in the Sapindales, while WETTSTEIN (158) puts it in the Tricoccac near the Euphorbiaceac. Since the woods of these two orders were not studied in this investigation, and since the study of the Buxaceac was based on such a poor representation of the family, no final deposition of the family is attempted at this time. However, the Buxaceac might very well be considered as being very close to the Hamamelidaceac.

PLATANACEAE

The Platanaceac are a family of large trees occurring in the eastern Mediterranean region to the Himalayas and in North America from Mexico to Canada. The single genus, *Platanus*, is divided into 6 species. The present study is based on an examination of the xylem of 3 species.

Growth rings are present in all. The fibrous tracheary elements are all tracheids.

The vessels are mostly solitary with some pore multiples. The wood is diffuse-porous. The vessels are thin-walled and angular (fig. 41) with a slight tendency toward rotundity in some vessels. The vessel diameters are very small to small, mostly small (range 46 to 94 μ ; mean 74 μ). Tyloses, present in all the species, are thin-walled and many. The perforation plates on the vessel members are scalariform and simple. On most of the plates the wide apertures are bordered at the ends and the bars are many. The vessel element end walls vary from 15° to 55°. Intervascular pitting is mostly opposite, with some alternate and rarely transitional. Vessel element length is medium sized (range 430 to 640 μ ; average 543 μ).

The rays are homogeneous I. They are from 1 to 15 cells in width. The uniseriates vary from 1 to 19 cells in depth and the multiseriates from 9 to several hundred cells. The rays are very wide, or so-called "compound," in all 3 species.

The xylem parenchyma is usually metatracheal, forming loose uniseriate lines (fig. 41). The parenchyma is rarely diffuse. Vessel-

parenchyma pitting is opposite and alternate, or less often transitional.

The anatomy of the Platanaceae resembles that of the Hamamelidaceae in many ways, yet the former is on a higher plane of anatomical specialization than is the latter. In the Platanaceae there is a tendency toward round pores, the vessels are larger, there are some simple perforation plates on the vessel members, there is some alternate intervacular pitting, the vessel elements are shorter, and the rays are homogeneous I.

The Platanaceae are also higher than the Stachyuraceae, Myrothamnaceae, and Buxaceae in all anatomical particulars.

This description of the woods of the Platanaceae does not differ very markedly from that of BRUSH (41).

EUCOMMIACEAE

The family Eucommiaceae contain the single species, *Eucommia ulmoides* Oliv. It is a tree of temperate China. The present study is based on 1 specimen with but 3 growth rings.

All the fibrous tracheary elements are tracheids. Vessel distribution is mostly solitary with a few pore multiples and clusters. There is a slight tendency toward ring-porosity in the wood. The pores are angular and thin-walled (fig. 42). The diameter of the pores is extremely small, rarely very small (range 20 to 34 μ ; mean 27 μ). All the perforation plates on the vessel elements are simple. The end walls on the vessel members vary from 20° to 60°. Intervacular pitting is opposite with some alternate. Vessel element length is very short to medium sized, mostly medium sized (range 230 to 400 μ ; mean 336 μ). There are spiral thickenings in the vessel members.

The rays are heterogeneous IIB, almost homogeneous I. The rays are uniseriate or biseriate. The former are 1 to 43 cells in depth and the latter from 4 to 32 cells.

The xylem parenchyma is diffuse and terminal (fig. 42). Vessel-parenchyma pitting is opposite and alternate.

The Eucommiaceae are higher than the Hamamelidaceae in that the former have vessel elements with simple perforation plates, there is some alternate intervacular pitting, the vessel elements are

shorter,⁶ and the rays are heterogeneous IIB. The Eucommiaceae also have a higher type of vessel element than the Platanaceae.

Anatomically the Eucommiaceae are almost on the same level of specialization as are the Ulmaceae. They differ from the Ulmaceae in that they have tracheids and the parenchyma distribution is diffuse and terminal. Some of the Ulmaceae have vestigial scalariform perforation plates on the vessel elements also.

HYDRANGEACEAE

The family Hydrangeaceae consists of shrubs and trees. Their geographical distributional areas are chiefly temperate North America and eastern Asia. ENGLER and PRANTL (62) place 16 genera and some 150 species in this group. The present study is based on an investigation of 3 genera and 11 species. The genera studied are *Philadelphus* (shrubs) (3 spp.) (very young), *Deutzia* (shrubs) (4 spp.), and *Hydrangea* (shrubs or at times trees) (4 spp.).

Growth rings are present in 3 species. The other species were represented by specimens too young to show the rings.

There are tracheids and fiber-tracheids in 8 of the species. Two of these species have spiral thickenings in the tracheids and in the fiber-tracheids. One species has septate fiber-tracheids exclusively.

The pores are mostly solitary with a few multiples (fig. 43). Two genera are diffuse-porous and one is ring-porous. The vessels are angular (fig. 43). The walls of the vessels are thin in 10 species, and thin to thick in 1 species. Vessel diameters are extremely small to moderate sized, mostly very small (averages range 22 to 130 μ ; mean of the family 55 μ). Tyloses are present in 1 species. Here they are thin-walled and few. The perforation plates on the vessel elements in all the species are exclusively scalariform. The plates have perforations with borders at the ends in 1 species and non-bordered in 10 species. All the species have many bars, except 1 species which has a few bars on the plates of vessel members. One species has wide perforations; all the others have narrow. The vessel element end walls vary from 5° to 65°. Intervascular and vessel-parenchyma

⁶Of course this study is based on young material; yet the vessel elements are so very much shorter than in the Hamamelidaceae that this statement is probably accurate.

pitting is scalariform and transitional in 1 species; scalariform, transitional, and opposite in 1; scalariform, transitional, opposite, and alternate in 3; and transitional, opposite, and alternate in 1. The vessel pits are small in 1 species. Vessel element length is medium sized to very long, mostly moderately long (averages range 560 to 1100 μ ; mean of the family 946 μ). Spiral thickenings are present in the tips or "ligules" of vessel elements in 5 species. Two of the latter species have spiral thickenings in the tracheids and fiber-tracheids also. Two species have spiral thickenings throughout the vessel elements; that is, not restricted to the tips.

The rays are heterogeneous I in 2 genera and heterogeneous IIA in 1. In width, the rays vary from 1 to 6 cells. In depth, the uni-seriates run from 1 to 26 cells and the multiseriates from 9 to several hundred. Some sheath cells are present in the rays of all 3 genera.

Xylem parenchyma distribution is diffuse in 1 species and diffuse and vasicentric in 4 others (fig. 43). Just a few cells are in contact with the vessels in the vasicentric type of parenchyma; that is, a continuous sheath is not formed about the vessels.

Anatomically the Hydrangeaceae are slightly higher than the Hamamelidaceae: most of the species have some fiber-tracheids, 1 form has septate fiber-tracheids, 1 species has thin to thick vessel walls, vessel diameter is larger, a few species have some alternate intervascular pitting, and the vessel elements are slightly shorter.⁷

GROSSULARIACEAE

The Grossulariaceae are a family of shrubs found in the north temperate zone, on the mountains of Central America, and on the Andes of South America. The family is made up of 1 genus, *Ribes*, and about 140 species. The present study is based on an investigation of 4 species.

Growth rings are present in all. There are tracheids about the vessels, but the rest of the fibrous tracheary elements are septate fiber-tracheids.

Most of the vessels are solitary, some are in multiples, and a few are in clusters. All the species examined are ring-porous. The vessels

⁷ Based on young material, however.

are angular and thin-walled (fig. 44). The vessel diameter is extremely small to very small, mostly extremely small (range 14 to 42 μ ; mean 28 μ). The perforation plates on the vessel elements are exclusively scalariform in 3 species and scalariform and simple in 1 (fig. 61). In the latter group the vessels with simple perforation plates are very rare. All the species have non-bordered apertures. The bars on the plates are intermediate in number in 2 species and few in 2 species (fig. 61). Half the forms have narrow and the other half have wide perforations. The vessel element end walls vary from 5° to 40°. Intervascular and vessel-parenchyma pitting is scalariform (most), transitional, opposite, and alternate (rare). Vessel element length is medium sized (range 360 to 660 μ ; mean 473 μ).

The rays are heterogeneous IIA. They are very wide, or so-called "compound," being from 1 to 22 cells in width. The uniseriate rays vary from 1 to 15 cells in depth and the multiseriates from 9 to 70 cells. Sheath cells are present in the rays of 2 species.

The xylem parenchyma is metatracheal, forming bands from 1 to 6 cells in width. These bands are quite rare, however.

The secondary xylem of the Grossulariaceae resembles very closely that of the Hydrangeaceae. The former is slightly more specialized in that all the species have septate fiber-tracheids, 1 species has some simple perforations on the vessel elements, the vessel member length is shorter, all the rays are IIA, and the parenchyma is metatracheal. In the Hydrangeaceae, however, the vessel diameter is greater and the vessel member end walls are not so oblique.

ESCALLONIACEAE

The Escalloniaceae are a family of shrubs or trees occurring mainly in the southern hemisphere. There are 22 genera and about 100 species in this group. The following anatomical description is based on a study of 4 genera: *Brexia* (young), *Itea* (young), *Escallonia*, and *Carpodetus*.

Growth rings are present in 2 genera. Tracheids and fiber-tracheids are found in all 4 genera.

The vessels are mostly solitary with just a few multiples. All the species are diffuse-porous. The pores are angular and thin-walled

(fig. 45). Vessel diameters are extremely small and very small, mostly very small (average range 18 to 39 μ ; mean of the family 30 μ). The perforation plates on the vessels are exclusively scalariform in 3 genera and simple with some vestigial scalariform in *Brexia*. In the 3 genera with scalariform perforation plates, the perforations are completely bordered in 1 genus and non-bordered in the other 2. All 3 genera have narrow apertures and many bars. The vessel member end walls vary from 5° to 55°. Intervascular pitting is scalariform, transitional, and opposite in 2 genera; transitional, opposite, and alternate in 1; and opposite and alternate in 1. The pits on the vessel elements are small in 3 genera. Vessel element length is medium sized to very long, mostly medium sized (averages range 445 to 1120 μ ; mean of the family 687 μ). Spiral thickenings are found in the vessel members of 2 genera.

The rays are heterogeneous I in *Carpodetus*, heterogeneous IIA in *Escallonia*, and heterogeneous III in *Brexia* and *Itea* (both young). In width, the rays vary from 1 to 7 cells. *Carpodetus*, however, has very wide, or so-called "compound" rays. In the latter genus the rays vary from 1 to 20 cells in width. In depth, the uniseriate rays run from 1 to 53 cells and the multiseriates run from 5 to several hundred (in *Carpodetus*). There are some sheath cells in the rays of 2 genera.

Xylem parenchyma is diffuse and metatracheal in 1 genus and diffuse, vasicentric (fig. 45), and metatracheal in 1. In both genera the metatracheal parenchyma is in the form of irregular, uniseriate lines. The vasicentric parenchyma cells usually do not form continuous sheaths about the vessels. The xylem parenchyma is metatracheal (1 to 6 cells wide) in *Brexia*. No parenchyma was observed in *Itea* (young). Vessel-parenchyma pitting is scalariform, transitional, and opposite in 2 genera and transitional, opposite, and alternate in the other 2.

Crystalliferous chambered parenchyma cells are found in *Brexia*. Crystals are present in the ray cells of *Brexia* and *Carpodetus*.

Anatomically the Escalloniaceae are very close to the Grossulariaceae. The former perhaps may be considered to be a little higher, for 1 genus in the Escalloniaceae has vessel elements with simple and rarely vestigial scalariform perforation plates. On the other

hand, the Grossulariaceae are more specialized in regard to certain other characters.

The 3 families Hydrangeaceae, Grossulariaceae, and Escalloniaceae are very similar anatomically, but in general it may be said that the Hydrangeaceae are primitive, Grossulariaceae less so, and Escalloniaceae least primitive.

RECORD (118) has described the wood of *Escallonia tortuosa* H.B. and K. It varies but slightly from *E. rubra* Pers. which was examined by the writer.

BRUNELLIACEAE

The Brunelliaceae are a family of trees inhabiting the Andes from Mexico to Peru. ENGLER and PRANTL (62) list 1 genus with 17 species in the family. The present study is based on an investigation of several specimens of *Brunellia comocladifolia* Humb. and Bonpl.

No growth rings are found. The fibrous tracheary elements are fiber-tracheids.

There are slightly more solitary pores than pore multiples. The wood is diffuse-porous. The vessels are angular and thin-walled. Vessel diameters are very small and small, mostly small (range 36 to 82 μ ; mean 62 μ). Tyloses are present and are thin-walled and few. The perforation plates on the vessel elements are scalariform and simple. The vessel elements with scalariform perforation plates have perforations bordered to the middle; the bars are intermediate in number and the apertures are wide. The end walls of the vessel members vary from 5° to 40°. Intervascular pitting is scalariform, transitional (fig. 59), and rarely opposite. The vessel elements are medium sized to very long, mostly moderately long (range 720 to 1480 μ ; mean 1093 μ).

The rays are heterogeneous I; some specimens have only uniseriate rays and so the rays would be classed as heterogeneous III. The rays are 1 to 6 cells in width. The uniseriates run from 1 to 45 cells in depth and the multiseriates from 4 to 35 cells.

No xylem parenchyma was observed in any of the specimens. The vessel-parenchyma pitting (to the ray cells) is scalariform, transitional, and opposite.

The Brunelliaceae are higher than the Hamamelidaceae in the

following ways: presence of fiber-tracheids, larger vessel diameter, and presence of some vessel elements with simple perforation plates. The Brunelliaceae are also higher than the Hydrangeaceae on the basis of these 3 characters. However, the latter have less oblique vessel end walls, some alternate intervascular pitting, shorter vessel elements, and a higher type of ray in some species.

CUNONIACEAE

The Cunoniaceae are mainly an Australasian family with 26 genera and about 250 species of trees and shrubs. Three genera and 12 species were examined in this study. The genera studied are *Ceratopetalum*, *Cunonia*, and *Weinmannia* (10 spp.).

Growth rings are present in 11 species. The fibrous tracheary elements in all the species are tracheids.

Most of the pores are solitary, with a few pore multiples and clusters in *Ceratopetalum*. In the other 2 genera the pores are mostly solitary with a few pore multiples. All the woods are diffuse-porous. RECORD (122) states that *Platylophus* is partially ring-porous. The vessels are angular and thin-walled in all species. The vessel diameters are extremely small to small, mostly small (averages range 38 to 69 μ ; mean of the family 53 μ). A few thin-walled tyloses are present in 2 of the genera. The perforation plates of the vessel elements are exclusively scalariform in 2 genera and scalariform and simple in *Ceratopetalum*. The apertures in the scalariform perforation plates are bordered at the ends in 2 genera and non-bordered in *Weinmannia*. There are many bars in 2 genera and intermediate in number in 1. The apertures are wide in 2 genera and narrow in 1. Vessel end walls vary from 10° to 60°. The intervascular pitting is scalariform, transitional, and opposite in 3 genera. Vessel element length is medium sized to very long, mostly moderately long (averages range 761 to 1048 μ ; mean of the family 925 μ).

The rays are heterogeneous IIA in 2 genera. However, they are almost heterogeneous I in *Weinmannia*. The rays are heterogeneous IIB in *Ceratopetalum*. In width, the rays vary from 1 to 4 cells. The uniseriate rays run from 1 to 26 cells in depth and the multiseriates from 4 to 38 cells.

Xylem parenchyma is metatracheal (1 to 3 cells in width) in

Ceratopetalum. In the other 2 genera it is diffuse and metatracheal (1 to 7 cells in width). Vessel-parenchyma pitting is scalariform and transitional in *Ceratopetalum* and scalariform, transitional, and opposite in the other 2 genera.

Chambered parenchyma cells with crystals are found in all the species.

The Cunoniaceae are on about the same level of anatomical development as are the Brunelliaceae. The former are lower in that all the species have tracheids, the vessel diameter is slightly smaller, and 2 genera have exclusively scalariform perforation plates. On the other hand, the Cunoniaceae have shorter vessel elements, the vessel member end walls are less oblique, and the rays are of a higher type.

DICHAPETALACEAE (OR CHAILLETIACEAE)

The Dichapetalaceae (or Chaillatiaceae) are a family of small trees and shrubs occurring in the tropics, mainly tropical Africa. ENGLER and PRANTL (62) list 4 genera and some 219 species in this family. The summary of the anatomy given here is based on a study of *Tapura cubensis* Griseb.

Growth rings are present. The fibrous tracheary elements are fiber-tracheids.

Most of the vessels are in multiples; there are some solitary pores and a few clusters. The wood is diffuse-porous. The vessels are angular and thick-walled. The vessel diameters are usually extremely small with some very small (range 30 to 52 μ ; average 41 μ). The perforation plates are scalariform and simple. There are about an equal number of plates in each class. The perforations on the scalariform perforation plates are non-bordered and wide. There is an intermediate number of bars on the plates. Vessel end walls vary from 10° to 50°. The intervacular and vessel-parenchyma pitting is alternate. The pits on the vessel elements are very small. Vessel element length is medium sized to very long, mostly moderately long (range 620 to 1150 μ ; average 895 μ).

The rays are heterogeneous I and from 1 to 2 cells wide. The uniseriates vary from 1 to 45 cells in depth and the multiseriates from 9 to 62 cells.

Xylem parenchyma distribution is metatracheal (uniseriate lines). The ray cells bear many crystals.

ENGLE and DIELS (60) and BESSEY (33) place the Dichapetalaceae in the Geraniales. WETTSTEIN (158) assigns the family to the Tricoccae near the Euphorbiaceae. Since the woods of these other groups have not been examined by the writer, and since the anatomical description of the Dichapetalaceae is based on such an inadequate sample of the family, no attempt is made to dispose of the family at this time. However, the Dichapetalaceae are on about the same level of anatomical specialization as are the Cunoniaceae and Brunelliaceae—perhaps just a little higher in a few characters.

The description of the wood given here is in accord with that published by RECORD (117).

ROSACEAE

The Rosaceae are an extensive family of trees, shrubs, and herbs divided into some 90 genera and into about 2000 species. They have a cosmopolitan distribution, but are found mainly in the north temperate zone. The present investigation is based on a study of 36 genera and 68 species. The genera studied include *Physocarpus* (shrubs) (young), *Neillia* (shrubs) (young), *Stephanandra* (shrubs) (young), *Spiraea* (shrubs) (young), *Vauquelinia* (shrubs and small trees), *Exochorda* (shrubs) (young), *Coloneaster* (shrubs) (2 spp.) (young), *Pirus* (trees and shrubs) (6 spp.), *Eriobotrya* (trees), *Photinia* (shrubs and small trees), *Pourthiaea* (shrubs), *Amelanchier* (shrubs and small trees) (2 spp.), *Mespilus* (shrubs or small trees) (young), *Rubus* (shrubs) (4 spp.) (some young), *Potentilla* (shrubs and herbs) (2 spp.) (young), *Fallugia* (shrubs), *Cowania* (shrubs), *Purshia* (shrubs), *Chamaebatia* (shrubs) (young), *Rosa* (shrubs) (6 spp.) (young), *Pygeum* (trees and shrubs) (2 spp.), *Prunus* (trees and shrubs) (10 spp.), *Prinsepia* (shrubs), *Chrysobalanus* (shrubs and small trees) (2 spp.), *Licania*, *Hirtella* (shrubs or small trees), *Parinarium* (trees) (4 spp.), *Acioa* (trees and shrubs), *Lyonothamnus* (trees), *Aronia* (shrubs) (young), *Chaenomeles* (shrubs) (young), *Cercocarpus* (trees and shrubs), *Crataegus* (small trees and shrubs) (2 spp.), *Heteromeles* (shrubs), *Micromeles* (trees) (2 spp.), and *Opulaster* (shrubs). The anatomical characters of the young ma-

terial were not tabulated, unless this material showed striking differences in anatomy from the other specimens.

Growth rings are found in 20 species. Two species, *Prunus caroliniana* Ait. and *P. domestica* L., have vertical intercellular canals of the traumatic type.

The fibrous tracheary elements in 3 genera are all tracheids. In 23 genera there are tracheids and fiber-tracheids—mostly tracheids in the greater number of genera. In 4 genera the tracheids and fiber-tracheids have spiral thickenings.

Vessels are mostly solitary, with a few pore multiples in all but 1 genus. In the latter there are mostly solitary pores with a few multiples and rarely clusters. Three genera and 1 species of *Rosa* have ring-porous woods. *Opulaster* and 4 species of *Prunus* have semiring-porous woods. The rest of the genera have diffuse-porous woods. The vessels are angular (fig. 46) in 12 species and angular to round (fig. 47) in 16 species. Vessel walls are thin in 27 and thick in 1 species. Vessel diameter is extremely small to rather large (fig. 47), mostly very small and small (averages range 21 to 218 μ ; mean of the family 70 μ). A few thin-walled tyloses are present in 3 species. The perforation plates on the vessel elements are simple, with very few scalariform plates in 17 species. In 14 of this class of 17 species, some of the plates are reticulate (fig. 60), or are modifications of the reticulate arrangement (fig. 58). BLISS (34), MAC-DUFFIE (106), and THOMPSON (142) have described and photographed these reticulate and modifications of reticulate plates in the Rosaceae. In 3 of the 17 species no reticulate plates are seen. In 2 species the plates are simple and vestigial scalariform and in 9 species they are all simple. In the forms with scalariform perforation plates, the perforations are completely bordered in 2 species and non-bordered in 13; the bars are intermediate in number in 1 and few in 14; and the apertures are narrow in 1 and wide in 14. Vessel element end walls vary from 10° to 90°. Three species have some vessel element end walls at 90°. The intervacular pitting is transitional (rare), opposite (some), and alternate (most) in 2 species. It is alternate in 7 and in all the other forms it is opposite and alternate. Vessel element length is extremely short to moderately long, mostly medium sized (averages range 193 to 757 μ ; mean of the family 487 μ). Spiral thickenings are present in the vessel elements

of 21 species. Three of these 21 species also have spiral thickenings in the tracheids and fiber-tracheids.

The rays are heterogeneous I in 3 species, heterogeneous IIA in 7, heterogeneous IIB in 9, heterogeneous III in 2, and homogeneous I in 5. The rays in the 5 forms with homogeneous I rays are nearly heterogeneous IIB. The rays vary from 1 to 8 cells in width. One form, *Rubus australis* Forst., has very wide, or so-called "compound" rays (from 1 to 50 cells in width). In depth, the uniseriate rays are from 1 to 42 cells and the multiseriates from 3 to 84 cells, except in 2 forms in which the rays run to many hundreds of cells. There are a few sheath cells in 1 species.

Xylem parenchyma distribution is diffuse in 2 species; diffuse and vasicentric⁸ in 10; diffuse, terminal (1 cell wide), and vasicentric⁸ in 2 (fig. 46); diffuse, metatracheal (in irregular uniseriate lines), and vasicentric in 5; metatracheal (1 to 4 cells wide) and vasicentric (mostly complete sheaths) in 5; and diffuse and confluent in 1 (fig. 47). Vessel-parenchyma pitting is opposite and alternate in 14 species and alternate in 9. Unilateral compound pitting is found in 4 species. Pith flecks are seen in 5 species.

Crystals are present in the ray cells of some species. Crystalliferous chambered parenchyma cells are found in 6 species.

Only a few (4) herbaceous stems were examined. However, EAMES (57, 59), SINNOTT and BAILEY (133), and JEFFREY and TORREY (89, 90) have published descriptions of the anatomy of other herbs in the Rosaceae. As a result of this work, it may be said that many of the herbs of this family are of the "continuous" type while some have discrete bundles.

The Rosaceae are more specialized anatomically than are the Cunoniaceae. This is apparent for in the Rosaceae there are fiber-tracheids, angular to round vessels, larger pores, some species with simple perforations on the vessel elements, some forms with vessel end walls at 90°, shorter vessel members, some alternate intervascular pitting, and higher types of rays.

CALYCANTHACEAE

The Calycanthaceae are a small family of shrubs occurring in temperate eastern Asia, in North America, and in tropical Aus-

⁸ Not in the form of sheaths, just 2 or 3 cells next to the vessels.

tralia. The family consists of 1 genus and 5 species. A young piece of *Calycanthus mohrii* Small was available for anatomical study.

Growth rings are present. The fibrous tracheary elements are fiber-tracheids.

The vessels are mostly in the form of multiples with some solitary pores. The wood is ring-porous. Vessels are angular and thin-walled. Vessel diameter is extremely small to very small, mostly extremely small (range 16 to 36 μ ; average 27 μ). All the perforation plates on the vessel members are simple. Vessel end walls vary from 10° to 65°. The intervacular and vessel-parenchyma pitting is alternate. Vessel element length is moderately short to medium sized, mostly medium sized (range 330 to 500 μ ; average 414 μ). Spiral thickenings are present in the vessel elements.

The rays are 1 to 2 cells wide and are heterogeneous I. The uniseriate rays vary from 1 to 35 cells in depth and the multiseriates from 5 to 30 cells.

Xylem parenchyma distribution is vasicentric, not in the form of a continuous sheath about the vessels, but just 2 or 3 parenchyma cells in contact with the vessels.

The Calycanthaceae are rather highly specialized anatomically. The perforation plates are simple, the intervacular pitting is alternate, the fibrous tracheary elements are fiber-tracheids, and the vessel elements are fairly short.⁹ Admittedly this anatomical description is based on too poor a sample of the family to allow any convincing generalization to be made on its relationship. It can be said, however, that the derivation of the Calycanthaceae from the more primitive members of the Rosaceae would not be inconsistent with the anatomical facts. Yet it must be remembered that certain groups in the Magnoliales (the Himantandraceae and Lactoridaceae) have simple perforation plates on the vessel elements, and alternate intervacular pitting also.

ARISTOLOCHIACEAE

The Aristolochiaceae are a family of herbs or woody vines distributed widely in both temperate and tropical parts of the world. ENGLER and PRANTL (62) list 7 genera and some 381 species in this

⁹ Based on young material, however.

family. The anatomical notes are based on a study of 4 species of *Aristolochia*. Two of these species showed no secondary tissues.

Growth rings are present. The fibrous tracheary elements are tracheids and fiber-tracheids.

The vessels are solitary, in multiples and in clusters. The wood is ring-porous in 2 species (fig. 48). The vessels are angular to round with thin to thick walls. Vessel diameters are small to large, mostly rather large. The perforation plates are simple. Some of the vessel element end walls are 90° . Intervascular pitting is alternate. Vessel element length is extremely short to moderately short, mostly very short.

The rays are homogeneous and up to 40 cells wide. These very wide rays dissect the stem into discrete bundles (fig. 48). The rays may extend from one internode to another.

The xylem parenchyma is vasicentric and metatracheal.

SOLEREDER (136) has described the anomalous structure of the axis in *Aristolochia triangularis* Cham. In this species the original vascular ring is split up and a fan-shaped stele is produced.

It is apparent that the Aristolochiaceae are very highly specialized herbs and vines. The vessel elements are very large and quite short, and the wood is ring-porous. The rays are exceedingly wide and very deep and so the stele is divided up into a number of discrete bundles.

Discussion

PHYLOGENY

Interpreting the anatomical data given for each family in the light of the lines of structural specialization discussed in the introduction, together with a consideration of the facts from floral and general morphology, the writer has come to certain conclusions concerning the phylogenetic relationships of the various orders and families studied in this investigation. These conclusions are summarized in figures 62 and 63.

Figure 62 is an attempt to depict the levels or planes of anatomical specialization, as well as the phylogeny of the several groups. The chart is divided into four zones, beginning at the bottom of the page with a zone containing the families with exclu-

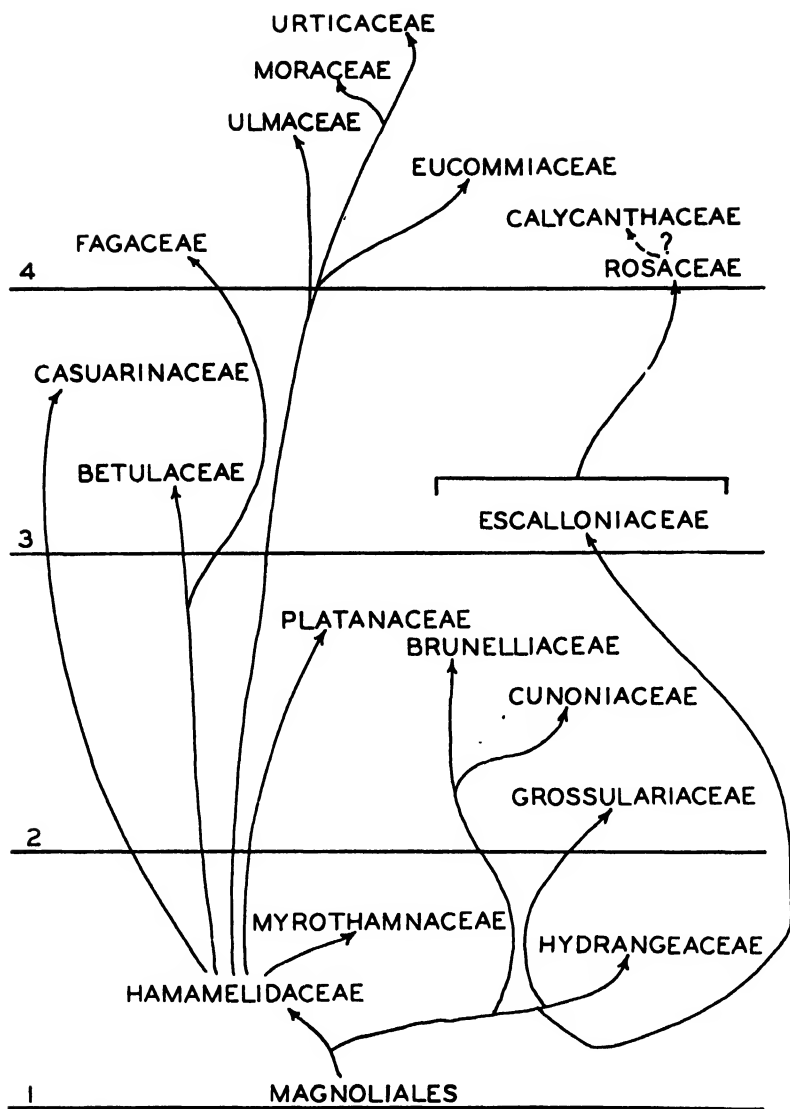


FIG. 62.—Proposed phylogenetic relationships of families investigated in this study: (1) exclusively scalariform perforation plates; (2) scalariform and simple perforation plates; (3) simple and vestigial scalariform perforation plates; and (4) simple perforation plates.

sively scalariform perforation plates on the vessel elements, next the zone or region with the families characterized by scalariform and simple perforation plates, still higher the zone containing the families with simple and vestigial scalariform perforation plates.

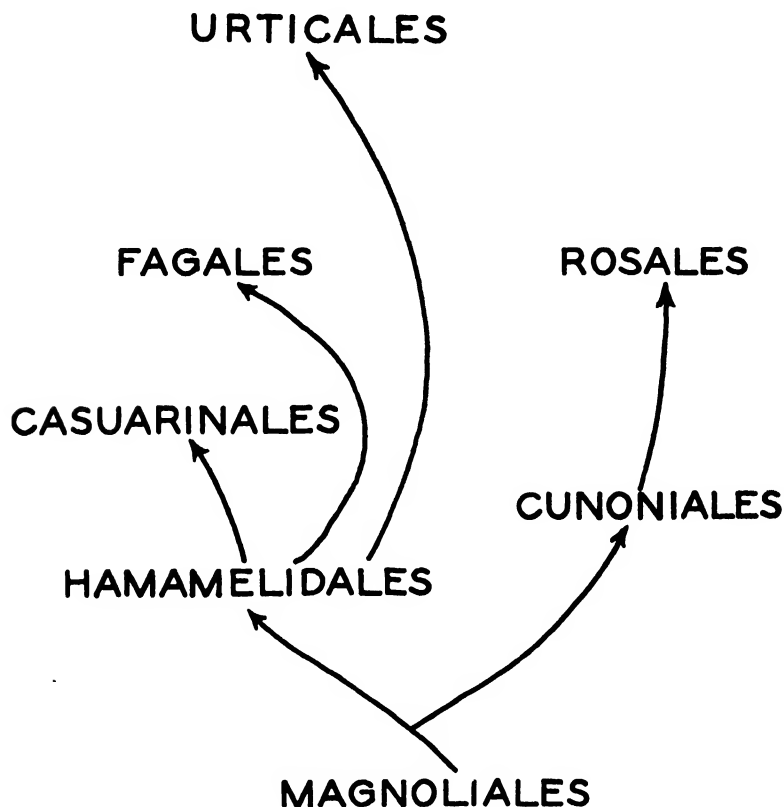


FIG. 63.—Proposed phylogenetic relationships of orders investigated in this study

and finally the region with the families which have simple perforation plates on the vessel elements. The relative positions of the families within the various regions are governed by the degree of anatomical specialization of the families. Thus two families may have exclusively scalariform perforation plates, yet one may have shorter vessel elements, a more primitive type of intervacular pitting, and a lower type of ray. This family, then, would appear

lower in the "exclusively scalariform" region than the other family. Some of the families, of course, exhibit considerable variation among the genera and species as to the type of perforation plate. In such cases the families are placed on the level of the highest members of the group. For example, in the Cunoniaceae certain forms have vessel elements with exclusively scalariform perforation plates, whereas certain other species have vessel members with simple and scalariform perforation plates; therefore the Cunoniaceae are placed in the region containing the families with scalariform and simple perforation plates.

The phylogenetic relationships expressed in these charts and in the text are not, of course, considered to be final. They merely represent the writer's conclusions based on the evidence at his disposal at the present time. Further evidence from the fields of cytology, paleobotany, anatomy, and taxonomy may alter considerably the conclusions reached in the present investigation.

Since in most cases the anatomical reasons for placing one family near or above another have already been stated in the summarizing paragraph at the conclusion of the anatomical description of each family, only a few supplementary comments on the anatomical evidence, together with a brief discussion of the facts from general external morphology, especially floral morphology, need to be added. Finally, the evidence from floral anatomy, paleobotany, nodal anatomy, and cytology will be discussed.

It has already been indicated that certain groups in the Magnoliales have a very primitive array of anatomical characters. The Magnoliales exhibit a considerable range of anatomical specialization, yet the more primitive groups (anatomically) may be characterized as follows: Some of the members have no vessels. Many of the forms have exclusively scalariform perforation plates. The perforations are completely bordered, or bordered to the middle, or bordered at the ends. Many species have many bars on the perforation plates; often the perforations are narrow. The vessel elements are characterized by great length and small diameter. The vessels are usually angular and thin-walled. The vessel end walls are very oblique. Intervascular pitting is scalariform in many. The woods are diffuse-porous. Most of the vessels are solitary. The fibrous

tracheary elements in many species are tracheids exclusively. The fibrous tracheary elements are very long. Many of the forms have heterogeneous I rays. Some of the species have diffuse parenchyma. Almost all forms are arboreal in habit. On the basis of anatomy, therefore, there is much to recommend the Magnoliales as a very primitive order among the dicotyledons. Likewise, from the standpoint of floral morphology, many phylogenists have considered the Magnoliales as the most primitive group, or among the most primitive groups of the flowering plants (31, 32, 33, 77, 79, 83, 3, 153). Those who regard the Magnoliales as a primitive group stress the following floral and external vegetative characters of that order: The flowers are hypogynous and hermaphroditic. The floral parts are often spirally arranged. There are many stamens which are usually free. There is much endosperm and the embryo is minute. The leaves are simple and usually alternate. ENGLER and PRANTL (61, 62) and WETTSTEIN (158), of course, dissent from the view that the Magnoliales are the most primitive order of the dicotyledons. WETTSTEIN thinks of the Magnoliales as being derived from some of the apetalous groups. ENGLER, in his later writings, feels that the Amentiferae and the Ranales (including the Magnoliales) are derived independently from a hypothetical group which he calls the Protangiospermae. Unlike WETTSTEIN, ENGLER does not say that the Magnoliales have evolved from the Amentiferae, but he does indicate that he believes that the Amentiferae possess more primitive characteristics than the Ranales. From the anatomical evidence, however, it would appear that the Magnoliales are primitive and the Amentiferae (at least the Casuarinales, Fagales, Juglandales, and Urticales) are more highly specialized.

Figure 62 pictures the Hamamelidaceae as a derivative of the Magnoliales. In the summary at the close of the anatomical description of that family it was indicated that the Hamamelidaceae are very close to the Magnoliales anatomically. Yet the Hamamelidaceae seemed a little higher. HALLIER (78, 79) derives the Hamamelidaceae from the Magnoliaceae. LOTSY (105) follows HALLIER in this respect. WETTSTEIN (158) also sees a relationship between the Hamamelidales and the Polycarpiceae (containing the Magnoliales). His system begins with the Amentiferae, however, and so he

places the Hamamelidales before the Polycarpicae. ENGLER and PRANTL and BESSEY picture the Rosales (containing the Hamamelidales) as coming from the Ranales, but they do not regard the Hamamelidaceae as the primitive family in the Rosales. It is obvious, then, that with the exception of WETTSTEIN, all those who do not derive the Hamamelidaceae directly from the Magnoliales, or Ranales (containing the Magnoliales), at least derive the Rosales (with the Hamamelidaceae) from the Ranales. In other words, from the standpoint of the flower there is nothing against the idea that the Hamamelidaceae came from the Magnoliales. The flowers of the Hamamelidaceae show the following advances over those of the Magnoliales: Often the flowers are unisexual, even dioecious. Sometimes the flowers are naked. The calyx at times is adnate to the ovary. The flower may be hypogynous to epigynous. The stamens are reduced in number. There are usually 2 carpels which are free at the apex. The ovules vary in number from many to 1.

Figure 62 indicates that the Myrothamnaceae had their origin in the Hamamelidaceae. Almost all phylogenists consider the Myrothamnaceae as being very close to the Hamamelidaceae. That the former are more advanced, or at least as highly advanced, as the more specialized members of the Hamamelidaceae is evidenced by the fact that in the former the flowers are dioecious, the spikes are catkin-like, the calyx and corolla are absent, and the stamens are reduced to 4-8.

The Platanaceae are clearly more advanced than the Hamamelidaceae anatomically. In all the systems the Platanaceae are placed in the immediate neighborhood of the Hamamelidaceae. Florally the former seem more advanced, or at least as highly advanced as the highest members of the Hamamelidaceae, in that the flowers in the Platanaceae are monoecious and in heads. The perianth is much reduced or may be lacking. The stamens are reduced to 3-8. There is usually 1, or rarely 2, ovules in the 1-celled ovary. The flowers are wind-pollinated.

It is obvious that in the families of the Hamamelidales (Hamamelidaceae, Myrothamnaceae, and Platanaceae) there are many tendencies which are very suggestive of the Amentiferac. Among these, mention might be made of the tendency toward unisexual

and naked flowers, the tendency to form heads and even catkin-like spikes, and the tendency to produce 2 carpels with 1 or 2 ovules. HUTCHINSON (84) has pointed out that the characteristic type of leaf common to many members of the Amentiferae is also found in the Hamamelidaceae.

No final deposition of the Stachyuraceae and the Buxaceae is attempted here for the reasons already indicated. However, no anatomical evidence was found which would militate against the placement of these two families in the Hamamelidales as derivatives of the Hamamelidaceae. Further study of the other suggested relatives of these two families may, of course, alter the situation.

The Eucommiaceae are much more specialized anatomically than are the Hamamelidaceae. In fact they are almost on the level with the Ulmaceae. In most of the systems of phylogeny, the Eucommiaceae are placed next to the Hamamelidaceae. WETTSTEIN, in the earlier editions of his *Handbuch der systematischen Botanik*, also classified the Eucommiaceae in the Hamamelidales. In his fourth edition (158), however, he puts the Eucommiaceae in the Urticales. H. HARMS, in the second edition of *Die natürlichen Pflanzenfamilien* (62), sees some characters of this family which remind him of the Hamamelidaceae and some which strongly suggest the Urticales. He concludes that "es lässt sich nicht leugnen, dass die Gattung (*Eucommia*) ebenso gut oder vielleicht noch besser in der Reihe der Urticales untergebracht werden könnte." As for external morphology, the flowers are dioecious and without a perianth. The carpels are 2 in number, 1 usually aborting. The ovules are 2 in number, pendulous and anatropous. The fruit is samaroid. There are latex tubes in certain parts of the plant. The Eucommiaceae are here regarded as being an offshoot from the main line of evolution in the Urticales, near the Ulmaceae. The Eucommiaceae are a very interesting and significant family, for they share many characters with the Hamamelidaceae on the one hand, and with the Urticales on the other.

Figure 62 depicts the Casuarinaceae as originating from the Hamamelidaceae. This is very possible as far as anatomical evidence is concerned, for the Casuarinaceae are higher than the Hamamelidaceae in every anatomical character. HUTCHINSON (83) derives

the Casuarinaceae from the Hamamelidales. In 1924 (82) and again in 1926 (84) he made clear that it was the Hamamelidaceae from which the Amentiferae originated. BESSEY (33) states that the Hamamelidaceae gave rise to the Casuarinaceae. PARKIN (112) likewise thinks that the Amentiferae may have been derived from the Hamamelidaceae. Indeed many of the floral characters of the Casuarinaceae are shared by some of the Hamamelidaceae. Among these characters, mention may be made of the monoecious and dioecious conditions, of the female flowers borne in heads, of the lack of perianth, and of the presence of but 2 carpels and 2 ovules. In addition, in such undoubted relatives of the Hamamelidaceae as the Myrothamnaceae, one finds male flowers in spikes and in the Platanaceae there is wind-pollination. On the other hand, ENGLER and PRANTL and WETTSTEIN consider the Casuarinaceae as very primitive dicotyledons. Essentially their arguments are four: presence of many megaspores, chalazogamy, wind-pollination, and absence of a perianth (or at most a bractlike perianth) in the Casuarinaceae. However, many megaspores have been discovered in the Ranales, in the Rosales, in the Fagales, and in other orders. Significantly, several megaspores have also been found in *Hamamelis*¹⁰ (131). Likewise chalazogamy has been found in many other groups. Indeed intermediate conditions between chalazogamy and true porogamy have been discovered, so that chalazogamy has lost much of its significance, for one may argue as have COULTER and CHAMBERLAIN (51) that chalazogamy is derived from true porogamy through such intermediate conditions as is found in *Ulmus*, *Cucurbita*, and *Alchemilla*. Further, ROBERTSON (128) has presented evidence to show that anemophilous flowers are derived from entomophilous ones. Lastly, the Casuarinaceae are considered primitive because they have no perianth, or at most a bractlike perianth. But it has already been indicated that certain members of the Hamamelidaceae have no perianth. Also, the Myrothamnaceae and Eucommiaceae have flowers with no perianth. The Platanaceae have

¹⁰ SHOEMAKER also found that fertilization took place 5 to 7 months after pollination in *Hamamelis*. This delay between fertilization and pollination, because of the similarity to the situation in the gymnosperms, is often cited as proof that some of the Amentiferae are primitive.

flowers with no perianth, or a much reduced one. In these families the perianth-less condition is usually considered a derived state. This appears to be true from the evidence obtained from studies in floral anatomy (36). If the lack of perianth in these families can be explained by reduction, there seems to be little to prevent the interpretation of the absence of a perianth in the Casuarinaceae in the same way. Future investigations of the floral anatomy of the Casuarinaceae no doubt will settle this matter. Certainly the highly specialized secondary anatomy of the stem would indicate a reduced rather than a primitive family.

Figure 62 indicates that the Fagales had their origin in the Hamamelidaceae. On the basis of secondary wood anatomy this seems quite likely, for the Betulaceae (anatomically the primitive family in the order) are more highly specialized than the Hamamelidaceae in all anatomical characters. HUTCHINSON derives the Fagales from the Hamamelidales. ARBER and PARKIN (3) are inclined to believe that the Fagales came from the Hamamelidaceae. Floristically the Fagales could have evolved from the Hamamelidaceae, for many of the characters of the Fagales are also seen in the Hamamelidaceae. Some of these characters are: monoecious flowers, rarely dioecious, or bisexual; calyx reduced or absent; stamens 2 to many; inferior ovary; 2 to 6 united carpels; and 1 to 2 ovules. Other characters of the Fagales are possessed by certain undoubted relatives of the Hamamelidaceae; that is catkin-like spikes. On the other hand, ENGLER and PRANTL and WETTSTEIN consider the Fagales as primitive largely on the basis of the simple perianth (sometimes absent), chalazogamy, and wind-pollination. This perianth may be reduced, however, as the floral anatomy indicates (2). Chalazogamy also seems to have lost much of its phylogenetic significance, as has been already suggested. Further, there seems to be good reason to believe that wind-pollination may be a derived condition. Finally, the anatomy of the secondary xylem of the stem is highly specialized. This evidence added to that from floral anatomy and that from floral morphology would seem to indicate that the Fagales are reduced and not simple plants. And the available facts at hand seem to point to their derivation from the Hamamelidaceae.

The Juglandales are not included in figures 62 and 63 for there

is good reason to believe that they, on the basis of their pinnate leaves, resinous condition, and other characters, might well have been derived from one of the groups in the Sapindales. Certainly the secondary xylem would indicate that the Juglandaceae are rather a high group and not a simple one as WETTSTEIN considers them. But since the xylem of the Sapindales was not studied in this investigation, no final deposition of the Juglandaceae is attempted at this time. However, HUTCHINSON, BESSEY, and HALLIER derive them from groups in the Sapindales.

Figures 62 and 63 picture the Urticales arising from the Hamamelidaceae, or at least some hamamelidaceous ancestor. From the anatomical viewpoint this origin for the Urticales is the most likely one of all the possible ancestors investigated, for the vestigial scalariform perforation plates in some members of the primitive family Ulmaceae indicate that the Urticales are to be derived from some group with scalariform perforation plates. ENGLER and PRANTL consider the Urticales as more or less primitive forms derived from the Protangiospermac. WETTSTEIN pictures them as arising close to the Fagales and Juglandales. He (157) does, however, see affinity between the Hamamelidaceae and the Urticales. This affinity is further borne out by the fact that he places the family Eucommiaceae, considered by nearly everyone else as close relatives of the Hamamelidaceae, in the Urticales. HUTCHINSON derives the Urticales from the Hamamelidales. GRIGGS (75) finds that the Platanaceae, considered by all to be very close to the Hamamelidaceae, resemble the Urticales in many ways. BESSEY derives the Urticales from the Tiliaceae and so considers them as members of the Malvales. HALLIER (77) considers them as derivatives of the Sterculiaceae through the Euphorbiaceae, the Rhamnaceae, or the Tiliaceae; subsequently he (79) changed his mind and derived them from the Terebinthaceae.

The writer has examined slides of the secondary xylem of 14 genera and 23 species of the Tiliaceae and 14 genera and 32 species of the Sterculiaceae. On the basis of this study, it is concluded that these two families did not give rise to the Urticales. The two families are very highly specialized anatomically. In the Sterculiaceae all the vessel elements have simple perforation plates. Twelve species are

found with storied structure. The pores are rather large in many species. The family also contains herbs and shrubs in addition to woody climbers and trees. In the Tiliaceae, all the vessel elements have simple perforation plates. Fifteen species have storied structure. Most of the species have libriform wood fibers. There are shrubs and herbs in this family also. The fact that some of the species in the Ulmaceae have vessel elements with vestigial scalariform perforation plates would seem to imply that this family was derived from some group with scalariform perforation plates.

The xylem of the Terebinthaceae has not been examined in this investigation, therefore it cannot be said with certainty that the Urticales are not derived from this family. LOTSY (105), however, in a critical analysis of HALLIER's system (which he favors for the most part), points out that HALLIER's derivation (78) of the Amentiferae (including the Urticales) from the Terebinthaceae is based on rather feeble evidence. Indeed HALLIER himself (79) made this very significant statement, after summarizing the evidence for deriving the Amentiferae and the Urticales from the Terebinthaceae: "*Malgré ces circonstances on ne doit pas encore pour le moment nier la possibilité d'une autre descendance des plantes à chatons. Mais il n'y a pas d'autres ancêtres possible que les Hamamelidacées, ou encore, . . . les Rosacées . . . La question pourra probablement être tranchée par l'étude comparée du bois*" (italics mine).

On the whole, HUTCHINSON's idea of tracing back the Urticales to the Hamamelidales seems to fit best with the anatomical data. From the standpoint of external morphology there is much to recommend this view. The following characters of the Ulmaceae, the primitive family in the Urticales, are shared by them and by some members of the Hamamelidaceae: The leaves are alternate and simple. The flowers are bisexual or unisexual. The calyx is 4- to 8-lobed and the lobes are imbricate. The ovary is superior with 1 to 2 united carpels containing 1 to 2 cells. There are 2 styles. There is 1 ovule, pendulous from near the top of the cavity of the ovary. The stamens are few in number, and the anthers which open lengthwise are 2-celled.

However, according to HUTCHINSON some of the Urticales are to be derived from the Aristolochiales. Presumably these are the

herbaceous members which are derived from the herbaceous side of his phylogenetic tree. Anatomically there is not the slightest evidence for this diphylysis of the Urticales. The Urticales are very homogeneous anatomically. The herbaceous forms are exactly like their woody relatives; or more correctly perhaps, like the first growth layers of their woody relatives, in all anatomical details. In the Urticaceae the herbs even have the odd heterogeneous I rays of their woody neighbors. *Urtica dioica* L. has even the unligified areas which occur in many of the woody members of the family. Apparently it would be stretching the concept of convergent evolution rather far in attempting to argue that the herbaceous forms coming from the Aristolochiales would be identical in the flower and even in the minutest details of anatomical structure with those woody forms derived from the Hamamelidales. The herbaceous species studied were clearly derivatives of the woody forms. Furthermore, there was no similarity between the anatomy of the Aristolochiales and that of the Urticales. Indeed, the Aristolochiaceae" (fig. 48) are much more specialized anatomically than are the herbs of the Urticales. The pores are larger, the vessel elements are shorter, and some forms are even ring-porous (fig. 48). The rays are very wide (fig. 48) in the Aristolochiaceae and so the herbs and vines are divided up into discrete bundles, whereas in the herbaceous forms of the Urticales the rays are narrow and the herbs are of the "continuous" stele type.

Figure 62 pictures the Ulmaceae as primitive, the Moraceae less so, and the Urticaceae as least primitive. Anatomically this is certainly true. With the exception of WETTSTEIN, the phylogenists likewise depict the same sequence of families. The evidence from external morphology also shows this sequence to be true. In the Ulmaceae the leaves are alternate. The flowers are perfect, polygamous, monoecious, or rarely dioecious. The calyx is subcampanulate and the lobes are from 4 to 9 in number. The stamens are equal to and opposite the calyx lobes, or there may be more stamens than the number of calyx lobes. The ovary is superior and made up of

"The Aristolochiales, according to HUTCHINSON, include the Aristolochiaceae, Cytinaceae, Hydnoraceae, and Nepenthaceae. But the last 3 families are obviously very specialized derivatives of the Aristolochiaceae and so their anatomy need not be considered.

2 carpels with 2 styles. There are 1 to 2 cells, usually 1, in the ovary. There is 1 anatropous ovule which is pendulous from the apex of the ovary. The seeds are without endosperm. The embryo is straight. In the Moraceae the leaves are alternate, rarely opposite. The flowers are monoecious or dioecious. The calyx is from 4- to 5-lobed—sometimes less, or lacking altogether. The stamens are equal to and opposite the calyx lobes. The ovary is superior usually and has 2 carpels, one of the latter not developing as a rule. The styles are 2 or 1. There is usually 1 ovule, sometimes 2, to each ovary. The ovules are anatropous and pendulous from the apex or rarely basal. The seeds are with or without endosperm. The embryo is often curved. In the Urticaceae the leaves are alternate or opposite. The flowers are monoecious, dioecious, or rarely polygamous. The calyx is 4-lobed, at times 5-lobed. The stamens are equal to and opposite the calyx lobes. The ovary is superior and is made up of 1 carpel with 1 cell. There is 1 style and 1 ovule. The ovule is "orthotropus" and erect at the base.¹² The seeds have endosperm mostly. The embryo is straight. It is apparent that in the 3 families there is a general trend from bisexual to monoecious to dioecious flowers, from many sepals to few, and from 2 carpels to 1. There is also a change in the position of the ovule.

ENGLER and PRANTL (61) seem to consider that the sub-family Moroideae are nearer to the Ulmaceae than are any of the other sub-families of the Moraceae. The tribe Fatoueeae of this sub-family, especially, is mentioned by them as being very similar to the Ulmaceae. It has already been stated that anatomically the Moroideae are on the whole the most primitive sub-family in the Moraceae. The tribe Fatoueeae also, on the available anatomical evidence, seems to have a good claim to being considered the most primitive tribe in the family, for the Fatoueeae have neither septate-fibers nor latex tubes in the rays.

As previously stated, the Rhoipteleaceae obviously do not belong in the Urticales. HANDEL-MAZZETTI (80) points out that this family differs from the Ulmaceae in that the Rhoipteleaceae have compound leaves and the ovule is not pendulous.

¹² The true nature of this ovule will be elucidated in connection with the discussion of floral anatomy.

Figures 62 and 63 show the Cunoniales (including the Hydrangeaceae, Escalloniaceae, Cunoniaceae, Brunelliaceae, and Grossulariaceae) arising from the Magnoliales. The Cunoniales in turn are shown giving rise to the Rosales (including the Rosaceae, and perhaps the Calycanthaceae). Almost all the phylogenists derive the Rosales (including the families of the Cunoniales¹³) directly from the Ranales. Likewise nearly all these writers picture the families here included under the order Cunoniales as primitive, and they depict the Rosaceae as derived. Since this is the accepted view among taxonomists, no detailed discussion of the floristics of the various families is necessary. Anatomically it can be said that the Cunoniales are primitive and the Rosales are derived. No attempt has been made to determine from which of the families in the Cunoniales the Rosales were derived. Trends toward the Rosales can be seen in the Cunoniaceae and in the Hydrangeaceae, Grossulariaceae, and Escalloniaceae. Indeed the last three groups could very well be placed in one family.

EVIDENCE FROM PALEOBOTANY

A survey of the paleobotanical literature (23, 24, 25, 26, 27, 28, 29, 30, 46, 97, 98, 103, 114, 161, 104, 92, 93, 140) pertaining to the families under consideration sheds but little light on the problem of phylogeny. The meager information which can be gleaned from this source is not convincing, for a good many of the families under investigation in the present study seem to have been already present in the rocks of the Cretaceous period. Further confusion results from the fact that many of the fossils in the Upper Cretaceous are merely leaves. As a result, a considerable proportion of these early records are subject to doubt and others are certainly erroneous. Accordingly only a brief summary will be made of the fossil evidence.

Arbitrarily, the families may be divided into three groups on the basis of their geological histories. The first group includes the families whose fossil records extend back beyond, or are abundantly represented in, the Upper Cretaceous. These families are the Magnoliaceae, Hamamelidaceae, Platanaceae, Fagaceae, Betulaceae, Juglandaceae, Moraceae, and Anacardiaceae. Many species

¹³ Most investigators—ENGLER and PRANTL, WETTSTEIN, BESSEY, and HALLIER—include the families of the Cunoniales in the Rosales.

of the Magnoliaceae, based on leaf material, are found in the Upper Cretaceous. There is some indication that this family was present in the Lower and the Middle Cretaceous periods. SAHNI (129) has described *Homoxylon rajmahalense*, a wood which resembles the homoxyulous Magnoliales (especially *Trochodendron*), in that there are no vessels, from the Jurassic of India. If this record is definitely established, it will greatly strengthen the position of the Magnoliales as a primitive angiospermous order (76). Both leaves and fructifications of the Hamamelidaceae have been removed from beds of the Upper Cretaceous. The leaves of Hamamelites are reported from the Mid-Cretaceous. LIGNIER (104) has described *Hamamelidoxyton* from the same horizon. Many leaves and a few fruits of *Platanus* (Platanaceae) are found in the Mid-Cretaceous. *Plataninium* (wood of the Platanaceae) has been described from the Upper Cretaceous. Leaves of many of the Fagaceae are present in the Upper Cretaceous. *Betula* leaves have been discovered in the same period but the other genera of the Betulaceae seem to make their first appearance in the Eocene. The leaves of *Juglans* (Juglandaceae) are found in the Upper Cretaceous, and there is a suggestion that they may be present in the Middle Cretaceous. Wood from this family under the name of *Juglandinium* is listed from the Upper Cretaceous. Fossil leaves of the Moraceae seem to be fairly common in the Upper Cretaceous. Without question many of these leaves have been incorrectly assigned to the genus *Ficus*, yet undoubted leaves and fruits of *Ficus*, and similarly leaves, fruits, and flowers of *Artocarpus*, are certainly found in this period. Some 10 to 12 species of *Rhus* (Anacardiaceae) have been described from the Upper Cretaceous.

The second group of families consists of those which are found in the Upper Cretaceous but are not abundant there. The Ulmaceae, Sterculiaceae, Sapindaceae, and Aristolochiaceae seem to belong in this category. The Ulmaceae are found in the Upper Cretaceous but are not so well represented as the families listed under the first group. The Sterculiaceae and the Sapindaceae have a few forms attributed to them from this geological horizon. Leaves and fruits of the Aristolochiaceae have been uncovered in deposits of the Upper Cretaceous.

The third group is made up of families whose earliest record is in

the Tertiary. The Casuarinaceae are mainly Tertiary, although one report places them as far back as the Mid-Cretaceous. The oldest known members of the Tiliaceae are from the Eocene. The Hydrangeaceae are first found in the Eocene. The Cunoniaceae appear in the Miocene and the Grossulariaceae occur in the Pleistocene for the first time. The Rutaceae have been found in the Lower Tertiary. Records of the Rosaceae are largely from the Tertiary. A few seem to extend back into the Upper Cretaceous. The Buxaceae have leaves and fruits in the Pliocene.

From the palaeontological evidence at hand it cannot be said with any degree of certainty that the Amentiferae or the Magnoliales are primitive. Representatives of both groups are seemingly present in the Upper Cretaceous. Yet BERRY (27) and THOMAS (140) have both called attention to the fact that the Magnoliaceae are very abundant in the Upper Cretaceous. SAHNI's discovery of the Jurassic *Homoxylon* seems to indicate also that the Magnoliales may with good reason be considered a primitive group from the standpoint of palaeobotany. However, it seems clear that if the Amentiferae were derived from some primitive angiosperm complex, this evolution must have occurred early. There is little in the available fossil evidence which would seem to oppose the notion that the Urticales, the Fagales, and the Casuarinales are derived from the Hamamelidaceae. Indeed the latter family seems to have a fair claim of being Mid-Cretaceous. Certainly it can be concluded that the palaeobotanical facts are in accord with the derivation of the Urticales from the Hamamelidaceae, rather than from the Anacardiaceae, or from the Sterculiaceae, or from the Tiliaceae.

It is odd that the Ulmaceae should appear to come later than the Moraceae. This situation is probably due to the rather questionable nature of some of the early records of *Ficus* leaves. Actually *Planera* (Ulmaceae) is found in the Upper Cretaceous and there are some reports of *Ulmus* from the same period.

The Cunoniales and Rosales seem to be definitely later than the Magnoliales.

Emphasis must again be placed on the fact that the fossil record is very incomplete, and therefore any conclusions based on this evidence are of doubtful validity. Future discovery and investiga-

tion of angiospermous fruits and wood from the Lower Cretaceous and Jurassic will no doubt remove much of the uncertainty.

EVIDENCE FROM NODAL ANATOMY

The research of SINNOTT (132) has shown that there are three fundamental types of nodal organization in the angiosperms. The primitive, or trilacunar condition, is one in which there is a foliar supply of three bundles, each causing a gap in the stem cylinder. Next there is the condition where a single gap is left by the foliar supply, whether the latter is single or multiple. This type of nodal structure is designated unilacunar, and has been derived from the trilacunar state, either through the fusion of the two lateral traces with the central one, accompanied by the disappearance of the separating segments of the stele, or by the abortion of the two lateral strands. Finally there is the type of nodal structure known as the multilacunar condition. Here the foliar supply consists of many bundles, each causing a gap in the cylinder. The multilacunar type is thought to be derived from the primitive trilacunar type by amplification.

SINNOTT (132) lists the following nodal conditions for the groups under consideration in the present study:

Magnoliales.....	3, 1, and many	Moraceae.....	3 and 5
Hamamelidaceae....	3	Urticaceae.....	3
Stachyuraceae.....	3	Eucommiaceae....	1
Buxaceae.....	1	Hydrangeaceae....	3 and (5 or 7)
Platanaceae.....	7	Cunoniaceae.....	3
Casuarinaceae.....	1	Brunelliaceae.....	3 and 5
Betulaceae.....	3	Rosaceae.....	3 and (1 or 5)
Fagaceae.....	3	Calycanthaceae....	3
Ulmaceae.....	3		

Other families which have figured in the discussion of the phylogeny of the groups investigated have nodal conditions as follows:

Anacardiaceae.....	3	Aristolochiaceae....	3
Juglandaceae.....	3 and (5)	Sapindales.....	3 and (5)
Tiliaceae.....	3	Rutaceae.....	3 and (1)
Sterculiaceae.....	3		

It can be seen that the proposed phylogeny of the various groups as pictured in figures 62 and 63 is in accord with the facts of nodal

anatomy. As SINNOTT remarks, the Ranales (including the Magnoliales) seem to have had the trilacunar condition primitively and the other types in that order were derived from this trilacunar condition. The Hamamelidaceae are trilacunar, and thus the various groups with the trilacunar, unilacunar, or multilacunar nodes could be considered to be derived from this family.

It must be admitted, however, that several of the other phylogenetic schemes receive as much support from nodal anatomy as does the one suggested in this paper. For example, from the evidence SINNOTT gives, the Urticales might be derived from the Sterculiaceae, or the Tiliaceae, or the Anacardiaceae. Taken alone, therefore, the evidence from nodal anatomy means little, but when it is placed beside the testimony of secondary xylem anatomy, of floral morphology, of paleobotany, and of other fields of research, it is not without import perhaps. Two facts stand out from the work on nodal anatomy, which even considered alone have some significance. First, the nodal organization of the Ranales is more variable than are most orders, and so SINNOTT has suggested that this variability or plasticity is an indication of the primitiveness of the Ranales. Second, the Casuarinaceae are unilacunar, and are therefore eliminated as a possible primitive group.

EVIDENCE FROM FLORAL ANATOMY

BECHTEL (19) has made an intensive study of the floral anatomy of the Urticales. As a result he found that in the flowers of the Ulmaceae there is evidence that an inner whorl of stamens and an inner whorl of perianth parts, supposedly petals, have been suppressed in the course of the evolutionary history of the family. (Also FAMES, 58.) He discovered conditions suggesting that formerly the carpels contained many ovules; also that there was slight evidence that the flowers of *Ulmus* might once have had more than 2 carpels. Anatomically the floral parts are spirally arranged; that is, the vascular traces to the floral parts are spirally arranged. He cites evidence which indicates that some of the perianth parts and some of the stamens have been suppressed in the 2 whorls which remain. The flowers are slightly zygomorphic, and BECHTEL suggests that this type of symmetry and the evidence of the multiovulate condi-

tion mean that the family had an entomophilous ancestor. The stamens are fused to the gamophyllous perianth. The number of floral parts varies from flower to flower in a given species. The male flowers of *Celtis* bear an abortive pistil, indicating derivation from a bisexual ancestor.

BECHTEL thinks that the Ulmaceae cannot be considered a very primitive group, therefore, for they show reduction in whorls, in members of a whorl, in the number of carpels, and in the number of ovules in a carpel. They are characterized by zygomorphy, syncarpy, the fusion of like parts (the sepals are fused to form the gamophyllous perianth), and the fusion of unlike parts (the stamens are fused to the perianth). On the other hand, he warns against placing the Ulmaceae too high, for the flowers have the spiral arrangement (anatomically) and the floral parts are variable in number.

In the present paper the Urticales are derived from the Hamamelidaceae. Therefore it will serve a useful purpose to test out the Hamamelidaceae as a possible ancestor of the Urticales with the anatomical facts reported by BECHTEL. Some members of the Hamamelidaceae possess an inner row of stamens and a corolla. Other forms have many ovules and are zygomorphic. Some of the species of the Hamamelidaceae have more than 5 sepals, petals, and stamens. Characteristically a calyx tube is present in the flowers. The flowers of the Hamamelidaceae are insect-pollinated as a rule. The flowers of the family do not have more than 3 carpels, but perhaps this loss of carpels took place in geological history at a time when the Hamamelidaceae and the Urticales had not become differentiated as such. Future floral anatomical studies of the Hamamelidaceae will reveal whether this family also has indications of previous polycarpous condition. There seems to be some doubt that these bundles in the Urticales actually prove the earlier existence of polycarpy (19, pp. 396, 400). All in all, the Hamamelidaceae seem to qualify as the ancestral group of the Ulmaceae.

In the Moraceae BECHTEL found that there were traces in the staminate flowers which indicated the earlier presence of a pistil. In other words, these unisexual flowers seem to be derived from bisexual ones and are not simple. In the forms with but 1 style he found vascular bundles of another.

In the Urticaceae there is only 1 carpel, but BECHTEL found vascular traces which imply the former presence of another carpel. He also found evidence of a pistil in the male flowers. Further, he settled the problem of the position and type of ovule in this family and in some members of the Moraceae. In the Ulmaceae and in some of the Moraceae, the ovule is anatropous and pendulous from the apex of the ovary. But in the other forms of the Urticales, the ovule is basal and "orthotropous," according to the usual interpretation. It has always been a problem to explain this basal, "orthotropous" ovule in a family which is supposedly higher than the Ulmaceae where the ovule is anatropous and pendulous. BECHTEL found that the vascular trace supplying the basal ovule goes up to the apex of the ovary and then bends down to the basal ovule. He therefore interprets this ascending and descending course of the trace to mean that the primitively pendulous and anatropous ovule of the Ulmaceae and some Moraceae has slid down to a basal position, and in this sliding down process the anatropous ovule has become erect to form a falsely "orthotropous" ovule.

BECHTEL concludes that from his studies the Ulmaceae are primitive, the Moraceae less so, and the Urticaceae least primitive. We have seen that this same sequence is indicated by the anatomy of the secondary xylem of the three families.

BOOTHROYD (36) has investigated the morphology and the anatomy of the flower and of the inflorescence of the Platanaceae. She finds that the perianth is much reduced. The calyx may be reduced to a mere cup, or may be lacking altogether in certain male flowers. The petals may be vestigial organs or absent. The male flowers have rudimentary carpels at times and the staminodia in the female flowers are clearly sterile stamens. The inflorescence is much reduced. All these facts point to the derivation of the flowers of the Platanaceae from bisexual, insect-pollinated flowers with well developed calyx and corolla. The Platanaceae are interesting for they resemble the Amentiferae in many ways, and they indicate how the flowers of the latter group may have lost their perianths and become unisexual in connection with modifications for wind-pollination.

There is nothing in BOOTHROYD's work which would militate against the derivation of the Platanaceae from the Hamamelidaceae

except the number of carpels. Since there are several free carpels in the Platanaceae and 2 (or rarely 3) fused carpels in the Hamamelidaceae, the former are not to be regarded as derivatives of forms like the modern Hamamelidaceae, but as derivatives of some common ancestor which possessed several free carpels.

ABBE (2) has investigated the floral and inflorescence anatomy and morphology of the Betulaceae. He found that the inflorescence is much reduced by loss of bracts and of florets. Fusion of bracts has also occurred. In the florets the perianth or perigon may be present, vestigial, or absent. If the perianth is lacking, traces indicating the former presence of tepals are found. The stamens may be reduced to 1.

ABBE's study shows conclusively that the Betulaceae are not simple plants but very much reduced ones. His work accords with the derivation of the Betulaceae from the Hamamelidaceae. He suggests that the ancestral condition for the gynoeceum in the Betulaceae might have been tricarpestry. Some of the members of the Hamamelidaceae have 3 carpels, so this condition is also satisfied by the Hamamelidaceae.

BERRIDGE (22) has studied the morphology and anatomy of the Fagaceae, and has found that in certain forms the female flowers bear stamens and the male flowers contain a rudimentary ovary. Also she found vascular conditions which suggest that the flowers of *Castanopsis* once possessed a whorl of petals. Some evidence was seen which signified that the tricarpestry ovary had been reduced from a pentamerous gynoeceum.

She concludes that the Fagaceae were probably derived from some primitive angiospermic stock with flowers characterized by bisexuality, syncarpy, multilocular ovaries (pentamerous possibly), and epigyny. These ancestral flowers had both calyx and corolla. On the basis of these facts she selects the Rosales (specifically the epigynous Rosaceae, or their near descendents) as the groups with closest affinity to the ancestors of the Fagaceae. Nothing in her work offers serious opposition to the derivation of the Fagaceae from the Hamamelidaceae, however, except the number of carpels. So it may be that the ancestral stock from which both the Hamamelidaceae and the Fagaceae evolved had more than 3 carpels.

MANNING (107) has made a brief report on his research on the Juglandaceae. He finds evidence of reduction in the inflorescence and in the flowers, and so concludes that the pro-juglandaceous stock had terminal panicles of perfect flowers.

In this connection it might be added that rudimentary ovaries have been found in the male flowers of some members of the Juglandaceae (83). Also, BENSON and WELSFORD (20) have published evidence that the ovule in this family is not basal and orthotropous, but is parietal and anatropous. As a result of these investigations, it seems clear that the Juglandaceae are much reduced plants.

EVIDENCE FROM CYTOLOGY

The haploid chromosome numbers¹⁴ of the groups investigated in the present study are as follows:

Magnoliales.....	14, 19, 38, 48
Hamamelidaceae . . .	12, 15, 24, 36
Platanaceae.	8, 10-11, 21
Casuarinaceae	12
Betulaceae.	8, 11, 14, 21, 28, 32, 35, 42
Fagaceae.....	11, 12, 13, 24
Ulmaceae.	14
Moraceae	8, 9, 10, 12, 13, 14, 15, 16, 28
Urticaceae.	7, 12, 13, 14, 16, 24
Hydrangeaceae	10, 11, 13, 14, 16, 18, 65
Grossulariaceae. . . .	8, 10
Escalloniaceae	11, 32
Rosaceae.....	4, 6, 7, 8, 9, 10, 14, 16, 17, 21, 26, 28, 32
Calycanthaceae	10, 11, 12
Juglandaceae.....	16, 17, 32
Aristolochiaceae. . . .	7, 14

The haploid chromosome numbers in certain groups which have figured in the discussion on phylogeny are as follows:

Rutaceae.....	8, 9, 13, 18, 35
Tiliaceae.....	8, 9, 41
Sterculiaceae.	8
Sapindales.....	5, 7, 11, 12, 13, 14, 15, 20, 26, 36, 40

¹⁴ These chromosome figures have been taken from GAISER, 67, 68, 69; TISCHLER, 144, 145; WANSCHER, 149.

WANSCHER (149) has shown that chromosome numbers in plants may be divided into five kinds of evolutionary series: "(1) The uniform series: for example 7-7-7-7. (2) The multiple series: 7-14-21-28, etc. (3) The descending series: 8-7-6-5. (4) The ascending series: 8-9, etc. (5) To the fifth class we may perhaps refer the uncertain and irregular secondary polyploid series; for example the series 7-14-17 in Pomaceae."

In the absence of other cytological evidence, it is difficult to determine whether one is dealing with an ascending or with a descending series in a given set of chromosome numbers. Also, the mere fact that two groups have the same chromosome number need not imply that the two are closely related. However, if evidence from floral morphology, stem anatomy, and other fields of research indicate a possible affinity between groups of plants, frequently the evidence from chromosome counts offers useful corroborative testimony. In this paper a certain phylogenetic scheme has been proposed; therefore it is interesting to see how the cytological facts fit in with the rest of the data.

It seems clear that the basic numbers, 12 and 15, of the Hamamelidaceae could have been derived from the 14 of the Magnoliales. Similarly the basic numbers of the Casuarinales, Fagales, and Urticales could be derived from the Hamamelidaceae by the methods WANSCHER has outlined.

Future cytological research on the morphology and behavior of chromosomes will no doubt establish the basic numbers in the various taxonomic units. Then it will be possible to make much wider use of these basic numbers in discussions of phylogeny. Even now a beginning has been made in this type of cytological investigation, but since the families have not as yet been studied extensively, it seems best to await further results.

General conclusions

As a result of the study of these families, it seems evident that in general the evolution of the flower has been correlated with the evolutionary development of anatomical structures. Thus it is that after the rearrangement of certain questionable groups, the classi-

fication of the taxonomist and the phylogenetic scheme of the anatomist show surprising agreement.

At this time it must be admitted that the use of the anatomical method in phylogenetic studies has certain definite limitations. BAILEY (12) has pointed out that similar combinations of anatomical characters may occur in families which are widely separated as to systematic position. He makes special mention of the remarkable resemblance between the xylem of *Maclura aurantiaca* Nutt. of the Moraceae and the wood of *Robinia pseudoacacia* L. of the Leguminosae. Therefore, if the anatomist were to build up a phylogenetic system on the basis of anatomy alone, some rather strange results would ensue. Because of this convergent evolution, and also because of parallel evolution in stem structures, the anatomist must proceed with caution.

The anatomist can, however, make certain definite contributions to problems of phylogeny if he uses the various systems built upon the basis of floral morphology as a background for his work. For example, in the present study we begin with the various placements of the Urticales of the several phylogenists before us. Then the theories of each phylogenist were tested out with the anatomical facts.

The suggestions of the anatomist can be of even greater value if he harmonizes his conclusions based on anatomy and floral morphology with the facts of paleobotany, floral anatomy, nodal anatomy, and cytology.

With the judicious employment of the methods outlined here, there is every indication that these methods will be of great value in the establishment of a natural classification of the angiosperms. Certain questionable groups may be assigned to their proper phylogenetic place. For example, the anatomy of the Rhoipteleaceae reveals that they do not belong in the Urticales. The compound leaves of the plants of this family lend support to this conclusion. A second type of service which the anatomist may render the phylogenist is assistance in the establishment of proper sequence of groups. HUTCHINSON, for example, shows the Magnoliales giving rise to the Cunoniales, the latter group giving rise to the Rosales, and finally

the Rosales giving rise to the Hamamelidales. Now the anatomical facts show that the Hamamelidaceae are very close to the Magnoliales, and so it is better to regard the Hamamelidales as being derived directly from the Magnoliales. Other lines of evidence seem to support this conclusion.

While not directly connected with the subject of the applicability of the anatomical method to problems of phylogeny, a number of other matters may be brought together at this time.

This survey of the anatomy of 22 families seems to lend support to the idea that non-septate fibrous tracheary elements (fiber-tracheids or libriform wood fibers) are primitive and the septate type of fiber (fiber-tracheid or libriform wood fiber) is derived. It was found that the former are usually present in structurally primitive woods, whereas the latter are present most often in rather highly specialized woods.

Since the solitary pore arrangement of vessels is found in primitive woods (such as those of the Hamamelidaceae), this solitary arrangement appears to be primitive, and the various aggregate arrangements, such as pore multiples, pore chains, and pore clusters, are derived types of vessel pattern.

There seemed to be some indication that the advent of ring-porosity, or of some factor or factors causing ring-porosity, gives an impetus to anatomical specialization. For example, in the Urticales the ring-porous woods of the Ulmaceae are more highly specialized than the diffuse-porous woods of the same family or of the Moraceae. Yet the Ulmaceae seem lower than the Moraceae from the viewpoints of floral morphology and floral anatomy. Anatomically too, the Ulmaceae on the whole seem less specialized than the Moraceae. If the ring-porous woods of the Moraceae are compared with the ring-porous woods of the Ulmaceae, the former are higher. Conversely, if the diffuse-porous woods of the Moraceae are compared with the diffuse-porous woods of the Ulmaceae, the former are higher. It will be recalled that BAILEY and TUPPER (18) found that the decrease in length of tracheary elements and the specialization of xylary structures was closely correlated with the advent and subsequent evolution of the vessel elements in the dicotyledons.

The anatomical investigation of the Moraceae and Urticaceae revealed that in comparative anatomical studies the secondary xylem of herbs and of shrubs must be compared with the secondary xylem of trees taken from a region close to the pith. If this is not possible, a correction must be made in the interpretation of ray type, vessel element length, vessel diameter, pore outline, and other structures in the essentially "youthful" herbs and shrubs.

Summary

1. There appears to be a correlation between the presence of sclerotic wood parenchyma cells in the xylem and the presence of sclerotic ray cells, sclerotic tyloses, and thick-walled fibers and vessel elements.

2. The advent of ring-porosity, or of some factor or factors causing ring-porosity, seems to give an impetus to anatomical specialization.

3. Non-septate fibers are more primitive than are the septate type of fibers.

4. The solitary pore arrangement of vessels is more primitive than the various aggregate arrangements, such as pore multiples, pore chains, and pore clusters.

5. In comparative anatomical studies, the secondary xylem of herbs and shrubs must be compared with the secondary xylem of trees taken from a region close to the pith.

6. The Hamamelidaceae are derivatives of the Magnoliales.

7. The Casuarinales, Fagales, and Urticales cannot be considered as primitive groups among the dicotyledons. These three groups are considered to be derivatives of the Hamamelidaceae.

8. The Cunoniales (including the Hydrangeaceae, Grossulariaceae, Escalloniaceae, Cunoniaceae, and Brunelliaceae) are derivatives of the Magnoliales. The Rosales have been derived from some group in the Cunoniales.

9. In the Urticales the Ulmaceae are most primitive, the Moraceae less so, and the Urticaceae are least primitive. The Eucommiaceae are also placed in the Urticales.

10. In the Moraceae anatomical specialization seems to have pro-

ceeded from the sub-family Moroideae to the Artocarpoideae to the Conocephaloideae to the Cannaboideae. The tribe Fatoucae of the sub-family Moroideae appears to be the most primitive one in the family.

11. There is no evidence for, and much against, the derivation of the herbaceous Urticales from the Aristolochiaceae.

12. The Rhoipteleaceae do not belong with the Urticales.

13. In general, HUTCHINSON's system is more in accord with the phylogenetic scheme proposed in this paper than are the other systems. His division of the dicotyledons into a herbaceous line and an arboreal line is not borne out by the facts revealed in the present investigation.

14. In general, the evolution of floral structures seems to be correlated with the evolutionary development of anatomical structures. After the re-arrangement of certain questionable groups, therefore, the classification of the taxonomist and the phylogenetic scheme proposed by the anatomist show striking agreement.

15. There is every indication that the study of anatomy will be of great value in the establishment of a natural classification of the angiosperms.

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DEVELOPMENT AND STRUCTURE OF THE WATERMELON SEEDLING

CONTRIBUTIONS FROM THE ILL BOTANICAL LABORATORY 493

GAYLE N. HUFFORD

(WITH FORTY-NINE FIGURES)

Introduction

BARBER (1) studied the fruits and seeds of the watermelon, *Citrullus vulgaris* Shrad. In addition to histological investigation, she determined that the cells of the embryo contain oil and protein granules as reserve food. Later, LANGELOD (7) compared the oil content of seeds of various crop plants and found that those of watermelon were highest in this respect, ranking above those of pumpkin and soy beans. Using root tips as material, WHITAKER (12) determined the chromosome number of several of the cultivated Cucurbitaceae. He reported eleven as the haploid number for *Citrullus*. CROCKER and associates (2) considered the peg to be a local overgrowth of the cortical cells, and demonstrated that such overgrowth is stimulated mainly by the arching of the hypocotyl. HOLROYD (4) made comparative histological studies of several of the Cucurbitaceae, particularly of the mature stems. He described *Citrullus* as "excelling all other members of the family in length of axis, luxuriant growth of side branches, and in size of hypocotyl and immediately connected regions above and below." He also considered the sieve tubes to be the largest of any cucurbits studied, those of the external phloem averaging twice the size of those of the internal. SLEETH (11) has shown that the formation of tyloses in *Citrullus* is incited by *Fusarium niveum*. Plants one to four months old showed an abundance of tyloses, the abundance being correlated with the quantity and nearness of the fungus. The evidence indicates that the formation of tyloses precedes or coincides with the spread of the fungus through the xylem. HOLROYD (4) and RUTLEDGE (10) have reviewed the extensive literature dealing with the nature of the bicollateral bundles in the Cucurbitaceae.

It is the purpose of this investigation to show some of the developmental and structural features of the early stages of the seedling.

Material and methods

Plants were grown in the field and in the greenhouse, and very young seedlings were germinated in moist cotton. Since the peg normally causes the hypocotyl to emerge at a sharp angle with respect to the primary root, it is difficult to obtain strictly transverse serial sections in the transition region. This difficulty was overcome by germinating the seeds in an erect position, micropylar end downward, and with the seed coats partially removed after the general method used by CROCKER (2). Most seeds so prepared were germinated in moist cotton, since it was easier to secure and maintain an erect position by wrapping the seeds in moist cotton pads and inserting the pads in shell vials. Some seeds so prepared were also grown satisfactorily in the greenhouse.

The best results for all young tissues were obtained when they were fixed in a Navashin solution made up as follows: (A) 10 cc. glacial acetic acid, 1.5 gm. chromic acid, and 90 cc. distilled water; (B) 40 cc. formalin and 60 cc. distilled water. Equal amounts of the two solutions were mixed just before using. Older tissues were fixed in formalin-alcohol.

Chloroform was used to clear all material to be sectioned. Cedar oil was used to clear seedlings for macroscopic study of the vascular system. Seedlings for this purpose were killed in formalin-alcohol and left in the solution until the chlorophyll had been dissolved from the tissues. They were then split along one side of the hypocotyl to the pith cavity, laid open, and clamped between two microscope slides. So held they were dehydrated in alcohol and cleared with cedar oil. Serial sections for the study of histogens of the root were cut at 6μ ; all others at 12μ . Flemming's triple stain was used.

Investigation

GERMINATION AND SEEDLING

The mechanics of germination are practically identical with those described by CROCKER (2) for other Cucurbitaceae. After the emergence of the primary root, the peg forms rapidly, so that by

the time the root is 2-3 cm. long it has fastened itself firmly against the lower half of the seed coats, while the simultaneous arching of the hypocotyl splits the halves apart. The opposing pull of the hypocotyledonary arch against the peg pulls the cotyledons from the integuments. These are left beneath the soil, often clamped over the peg. Rapid growth of the lower hypocotyl forces the arch upward through the soil and above ground. Continued growth results in the cotyledons being rapidly pulled from the soil. The arch straightens out and the cotyledons spread apart. Although yellowish or white at the time of emergence, the cotyledons and the aerial portions of the hypocotyl develop chlorophyll. It is only when seeds are planted on the micropylar end that the seed coats are carried above the ground by the cotyledons.

The time required for germination varies greatly. The seeds are very sensitive to temperature and water conditions. In warm greenhouse conditions the radicle usually emerges within 36-48 hours, and the cotyledons will be out of the ground and spread apart within four days. Growth of the hypocotyl is rapid. Within eight to ten days from time of planting it will have attained a length of 9-11 cm., which is about its maximum in an optimum environment. During this time there is a correspondingly rapid growth of the primary root and its branches, and of the cotyledons. The latter are ovate and have long and short diameters of about 11 and 7 mm. at the time they appear above the soil. Within a few days they reach maximum size, the average long and short diameters being 4 and 2 cm. respectively. These data relate to greenhouse-grown plants; under field conditions the periods of growth will be lengthened in proportion to the departure from optimum atmospheric conditions.

Full expansion of the cotyledons marks a definite stage in the growth of the plant, since two weeks to a month may elapse before further appreciable lengthening of the axis occurs. This stage is arbitrarily referred to as the seedling, of which this study covers only the earlier stages; that is, before marked secondary thickening has taken place.

At the time the cotyledons first spread apart the epicotyl is hardly visible. The point of the first regular leaf is deeply set in the angle between the cotyledons. The latter are densely spinulose

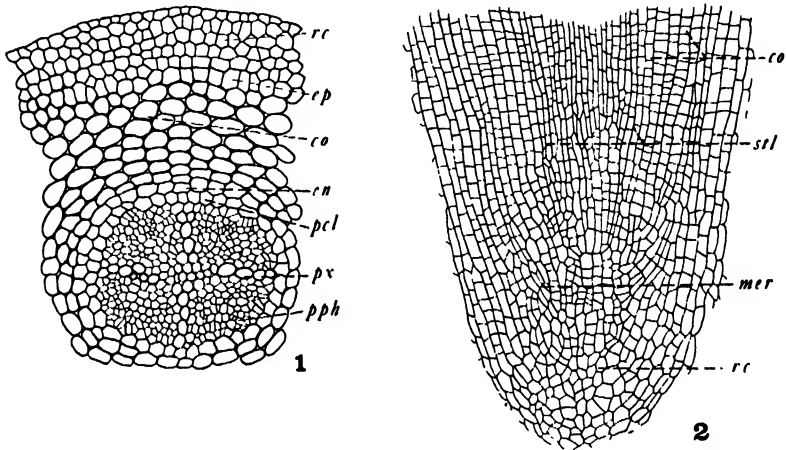
above and glabrous beneath, and each has seven main veins, more prominently displayed on the lower surface. The hypocotyl is somewhat flattened in the plane of the cotyledons and tapers slightly as it approaches them. It ranges from 3 to 4 mm. in diameter just above the peg to 1-1.5 mm. just below the cotyledons. Its surface is beset with spinulose hairs which increase in number and size from the ground upward. The primary root grows rapidly and apparently continues to do so throughout the life of the plant. It tapers abruptly just below the peg, and from it arise many laterals, definitely four ranked.

PRIMARY ROOT AND TRANSITION

The primary root is tetrarch exarch. Its general organization is evident early in the ontogeny of the axis. Figure 1 shows a transverse section less than 1 mm. from the tip of a very young seedling in which the primary root had attained a length of approximately 5 mm. The protoxylem has begun to differentiate and shows as a cross within the pericycle. The primary phloem is somewhat more advanced in its development. In fact it is characteristic that the phloem precedes the xylem in development, and also exceeds it in amount formed. The pericycle is uniformly one cell thick opposite the phloem, except that there is usually an extra layer two to four cells wide adjacent to the protoxylem. The cells of the pericycle are large and angular in cross section. The endodermis can be determined by position only, since its cells approximate the size and shape of the cortical cells. No Casparian strips are visible. The cortex is from eight to ten cells thick. The cells are very large, oval, and somewhat compressed periclinally. The intercellular spaces are many. The epidermis is composed of one layer of cells, which are more or less rectangular in cross section. Around the epidermis at this level there are four or five layers of the root cap which have not been abraded. The cells resemble those of the epidermis, and compactly surround them. With the exception of the outer cap cells, all are highly meristematic, as is evidenced by their thin walls, prominent nuclei, and dense cytoplasm. The protoxylem is becoming definitely differentiated.

Figure 2 shows a median longitudinal section through the root

tip. As indicated, there are no definite histogens. At the apex is a common meristematic region from which all tissues are derived. This meristem lies in a horizontal plane at the base of the central cylinder, but outward from the cylinder it is turned up slightly, so that it has the shape of a shallow bowl. All stelar and cortical tissues are derived from cells cut off to the inside of this bowl-shaped promeristem. Cells cut off to the outside form the calyptra. Cells are cut off centripetally from the upturned edges of the meristem.



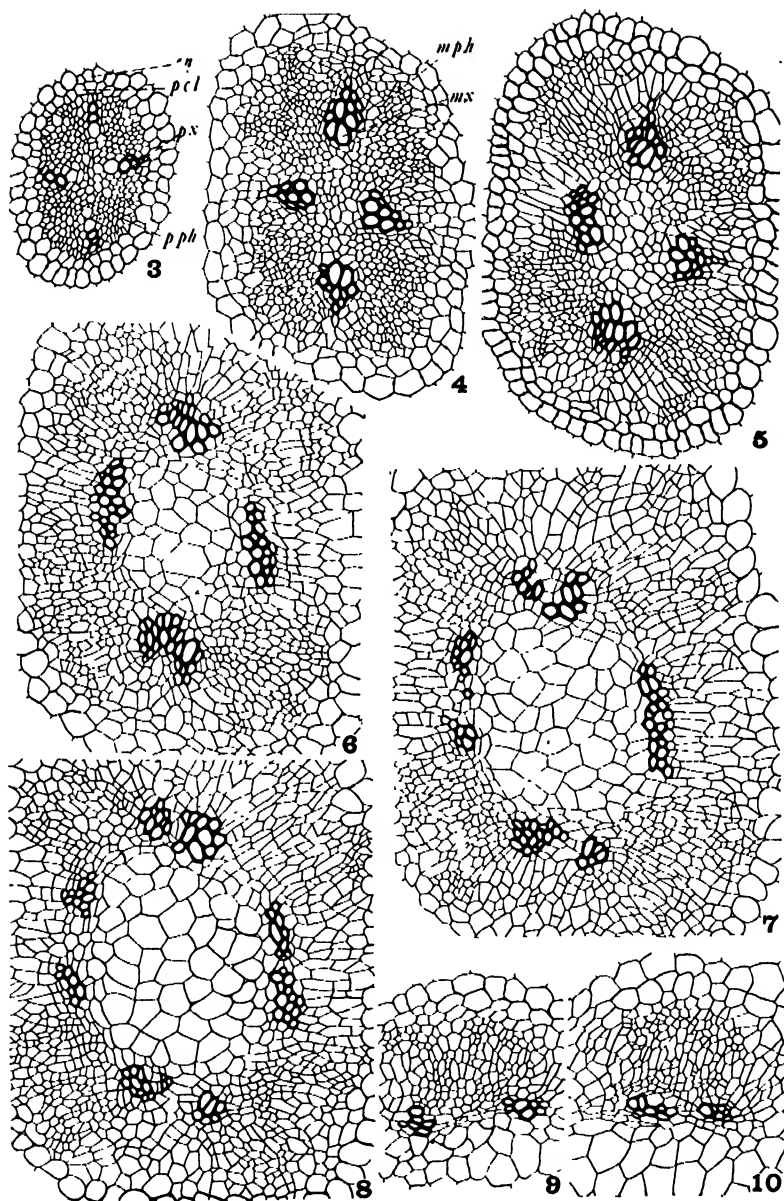
FIGS. 1, 2. —Sections from meristematic region of primary root: Fig. 1, cross section 2 mm. back from tip showing early differentiation of tissues (*rc*, root cap; *ep*, epidermis; *co*, cortex; *en*, endodermis; *pcl*, pericycle; *px*, protoxylem; *pph*, protophloem). Fig. 2, median longitudinal section through tip showing nature of unorganized meristem (*stl*, stele; *mer*, meristem). $\times 27$.

Because of this the cap does not break away from the root, but adheres closely to it for considerable distance. Continued, although irregular, breaking away of the cap cells eventually leaves the innermost layer of these cells, which were cut off externally from the meristem, as the epidermis. As a consequence of its irregular method of derivation, the epidermis usually presents a rather uneven surface instead of the smooth and even surface of an epidermis derived from a definite dermatogen. This meristematic region of *Citrullus* is similar although not identical with the type of meristem described by JANCZEWSKI (5) as being common among the Cucurbitaceae and the Papilionaceae. He considered the upturned outer limits of the

meristem the transverse generating layer from which the central cylinder and the cortex are derived to the inside, and the calyptra to the outside. He also described the manner in which the epidermis is developed. The description given thus far relative to figures 1 and 2 applies to the tip region regardless of the age or the length of the root. In slightly older roots, 15–20 mm. long, it is possible to follow the development of the primary root by examining identical structures at successively higher levels.

Figure 3 shows a section about 8 mm. back from the tip. The protoxylem is well differentiated, being composed of annular and spiral elements. The metaxylem has begun to differentiate centripetally from the protoxylem. The primary phloem shows little greater differentiation. The elements of each of the four groups tend to be divided into two smaller groups toward the adjoining protoxylem points, with prominent parenchyma cells between each group. There is no marked change in the pericycle. The endodermis is well marked by Casparian strips, which are more prominent here and in immediately adjoining regions than at any other level. There are no further changes in the cortex or epidermis, except increase in length. The length of each cell eventually becomes eight to ten times its width. Ultimately both are abraded. This loss begins early and proceeds gradually and irregularly. Apparently no cortical periderm is formed. Study of mature roots indicates that a periderm is formed by the pericycle as it becomes exposed by the final disappearance of the cortex.

Figure 3 shows a section still higher up, about 10.5 mm. from the tip and 4.5 mm. from the peg. The primary xylem has been augmented by the maturation of metaxylem elements centripetal to the protoxylem, which shows signs of being crushed out. The metaxylem consists of tracheids and large scalariform and scalariform-reticulate tracheae. There has been considerable addition to the primary phloem by the continued differentiation and maturation of metaphloem. Its elements are somewhat more specialized, sieve tubes and companion cells being present in addition to parenchyma. No crushing of the primary phloem was observed in any seedling material. In this respect, the functional persistence of the primary phloem, the situation is apparently similar to that reported for the

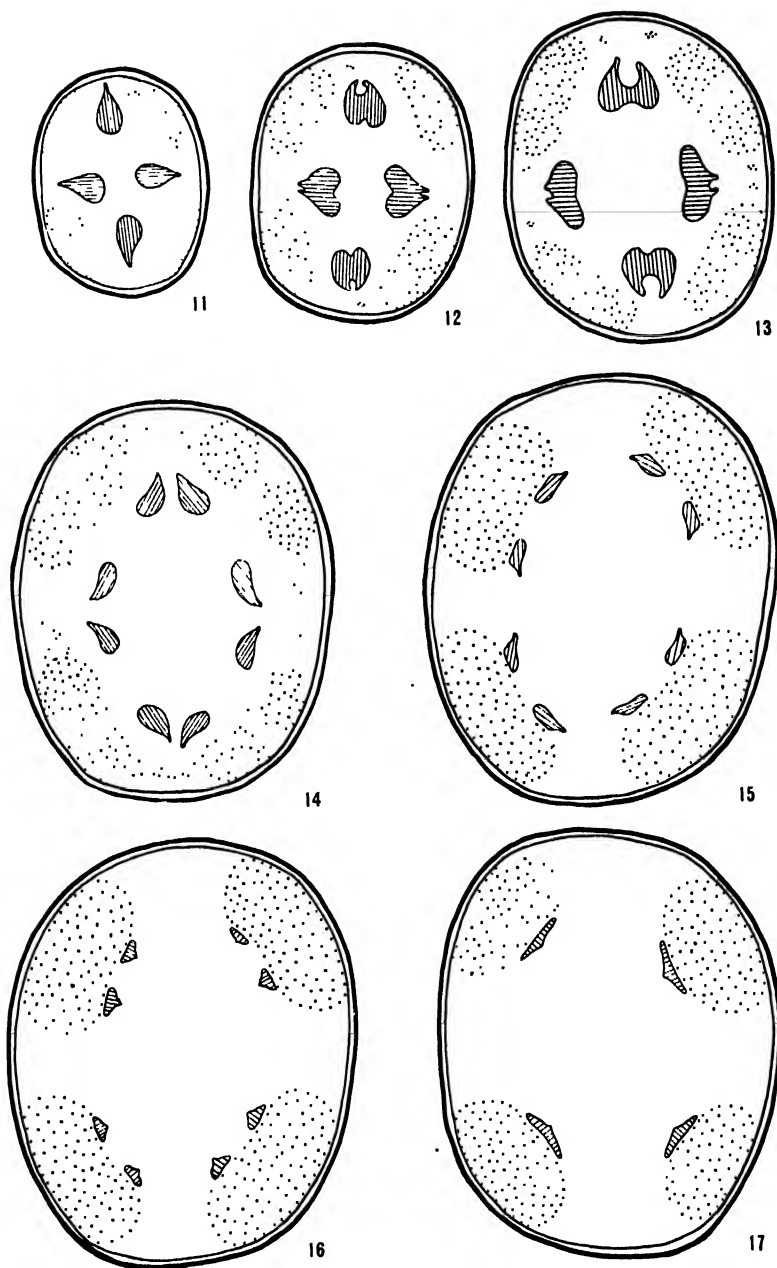


FIGS. 3-10.—Transverse sections from primary root about 15 mm. long showing differentiation of metaxylem and metaphloem and critical stages of transition. Figs. 3-5, stele and endodermis; figs. 6-8, stele, including pericycle; figs. 9, 10, one transition bundle only (*en*, endodermis; *pcl*, pericycle; *px*, protoxylem; *p ph*, protophloem; *mx*, metaxylem; *m ph*, metaphloem). $\times 27$.

genus *Cucurbita* (3). The pericycle of this and of succeeding levels is irregularly one to three cells thick, the extra layers being particularly noticeable opposite the protoxylem. Casparian strips are still prominent. The pith is more extensive than at lower levels, owing more to the increase in size of its cells than to their increase in number. The pith is present throughout the length of the primary axis. The combined proliferations of the various tissues and the growth of cells have practically doubled the width of the stele at this level as compared with the one just described. This rapid flaring of the primary root continues up to its mergence with the hypocotyl at the level of the peg.

Figure 5 represents a section 2.5 mm. above the one just described. The most notable change in the primary xylem is the much greater width of each of the four groups, due to continued differentiation of metaxylem laterally. There is evidence that each xylem group is about to diverge radially into two groups. The phloem groups are also much wider, owing to the greater number of parenchymatous cells between the smaller units within each large group. Small clusters of phloem elements are also sparsely scattered in the rays opposite the xylem. Sieve tubes and companion cells are well differentiated in the phloem at this level. The Casparian strips are not so distinct, being most prominent opposite the phloem.

Beginning here also there is rapid reorientation of the vascular bundles from the exarch and radial alignment of the root to the endarch and collateral alignment of the stem (figs. 11-17). First there is a radial separation of each xylem group to form two groups. These new groups apparently are diverged with the metaxylem ends turned away from each other and swung centrifugally through arcs of 180° . At about the time the protoxylem is lateral in position, the two sub-groups of phloem within each large group are far enough apart exactly to subtend the reoriented groups of xylem. This divergence of the phloem is sufficient to separate, almost if not quite, each phloem group into two distinct groups. At this time there are apparently eight bundles, therefore, but these are evidently in four closely aligned pairs. The continued lateral development of the xylem groups results in the members of each of the four pairs coming together to form four endarch collateral bundles, com-



FIGS. 11-17.—Diagrammatic drawings, based on figs. 3-10, showing nature of transition. Outer heavy line represents endodermis; inner line, pericycle; dotted areas, primary phloem; and lined areas, primary xylem.

pleting transition. Finally there are as many bundles as at the beginning. The phloem of each new bundle is that of one of the original groups, while the xylem is related to two bundles, half of each being continuous with the lateral half of two of the original bundles. These two strands maintain their entity for a time, but there is less parenchyma differentiated between them so that there is soon but one compact group. This final alignment of the four endarch bundles occurs at the lower level of the peg, scarcely 2 mm. above the level of the beginning of transition.

There are other important histological situations in the transition zone which are not particularly related to transition itself. These are indicated in the diagrams and in the detailed drawings made of sections taken at critical levels. Figures 12-14 show the presence of phloem across the rays opposite the xylem, while figures 15-17, taken at higher levels, do not show it. Figures 3-10 are of corresponding portions, as indicated in the diagrams. More and more sieve tubes and companion cells, separated by small groups of parenchyma, are differentiated, so that eventually there are no rays, but a continuous band of phloem. This is of considerable physiological importance, since it gives every portion of the root a connection with food channels so long as even one bundle retains its food conducting ability. A second important fact is that the tissues in the upper transition regions (at peg level) are more juvenile or nearer the embryonic condition than are those farther down the axis. This is particularly noticeable in the relative maturity of the xylem elements. This retention of meristematic activity is related to the rapid growth of the lower portions of the hypocotyl, especially during germination.

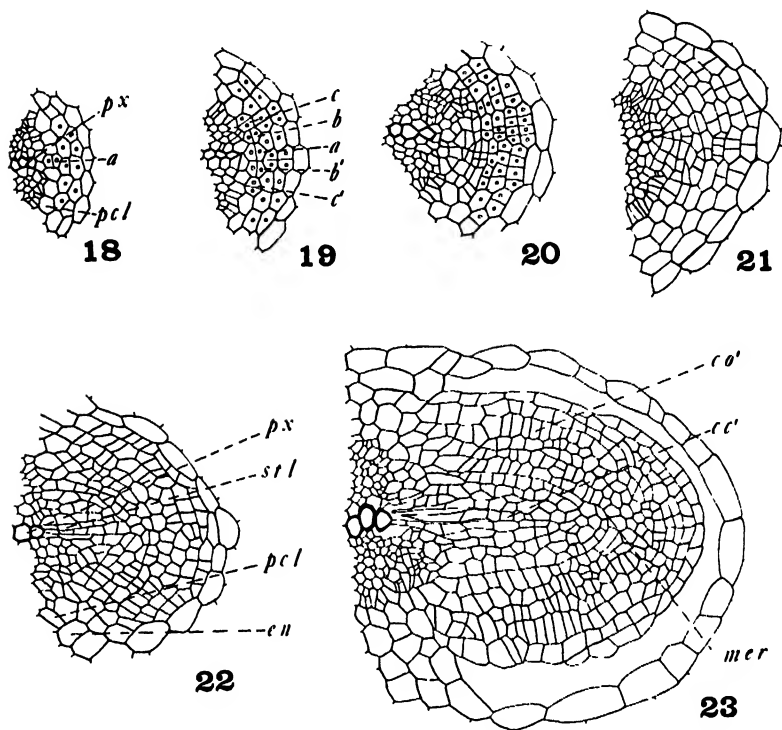
ORIGIN OF SECONDARY ROOTS

In addition to the abundance of secondary roots, they are characterized by their very early appearance from the viewpoint of ontogeny, and by the unusual manner of their origin. In general, the manner of origin agrees with that described by JANCZEWSKI (6). LE MAIRE (8) confirmed the work of JANCZEWSKI and elaborated somewhat on the mechanics of development in the different types of origin of secondary roots. Both considered this particular type

to be peculiar to the cucurbits and the legumes; both agreed as to the general plan of development; and both found considerable difficulty in tracing the exact procedure because of the tardy differentiation of primary tissues in the secondary roots. They concluded that the origin and nature of the meristem of the secondary root is as unusual as that of the primary root. Briefly they characterized this type as follows. Only the central cylinder of the secondary root arises from the pericycle of the primary; the remaining tissues are derived from the endodermis and the inner layers of the cortex.

Figures 18-23 are from cross sections of the primary root taken from levels successively back from the apical meristem, and are selected to show the activities of various tissues of the primary root in the development of secondary roots. Figure 18 shows a single cell of the pericycle which has divided tangentially. It is exactly opposite a protoxylem point. At the time this division occurs the protoplasm is dense within the adjoining cells of the pericycle, as well as in the surrounding cells of the endodermis and proximal cells of the immediately adjoining cells of the cortex. Figure 19 shows cells on either side of the initial divided, also tangentially, while the one next to each of these has divided radially. These two cells, which divide radially, mark the limits of the pericycle which enters into the formation of the secondary root. In addition several cells of the endodermis have divided tangentially, and some of the cells so derived have divided radially, thus outlining the beginnings of the secondary root in the cortex of the primary. Likewise radial divisions have occurred in the two or three layers of pericyclic cells previously mentioned as occurring opposite the xylem points. JANCZEWSKI (6) refers to these inner cells, together with those of the main layer of the pericycle, as pericambial, because of their relation to the formation of secondary roots. Figure 20 shows continued proliferations of all the tissues and also divisions beginning to occur in an adjacent layer of the cortex. Figure 21 shows, in addition to continued divisions, the beginning of demarcation of the central cylinder of the lateral root. In figure 22 this demarcation is still more definite. The stele of the secondary root is also accentuated by cells which form the xylem connections between the secondary root and the

xylem strand opposite which it is formed. Here too is evidence of the formation of the terminal meristem of the lateral root. Because



FIGS. 18-23.—Cross sections from primary root showing development of secondary roots: Fig. 18, single cell (*a*) of pericycle divided transversely to become a secondary root initial; other nucleated cells are results of divisions of endodermal cells (*px*, protoxylem; *pcl*, pericycle). Fig. 19, two pericyclic cells (*b*, *b'*) adjoining the initial have divided tangentially and two cells (*c*, *c'*) adjoining these have divided radially; all other nucleated cells still of endodermal origin. Figs. 20-21, continued development of secondary root tissues through proliferations of pericyclic and endodermal tissues. Fig. 22, stele of secondary taking form and inner cortical layers of primary contributing to outer tissues of secondary (*pcl*, pericycle of primary; *en*, endodermis of primary; *co*, cortex of primary; *stl*, steler tissues of secondary). Fig. 23, secondary ready to emerge through last cortical layer of primary (*cc'*, central cylinder of secondary, developed from pericycle of primary; *co'*, cortex of secondary, developed from endodermis and inner cortex of primary; *mer*, developing meristem of secondary). $\times 35$.

of the extent to which various tissues of the primary root have contributed to the formation of the secondary, it is difficult to recognize

with exactness the origin of the terminal meristem of the latter. JANCZEWSKI assumed that finally it is derived partly from the terminal cells of the central cylinder and partly from the cells, of endodermal origin, which surround the tip of the central cylinder. I was unable to determine from the material studied the exact origin of the cells in this meristematic region. From such as were examined, as illustrated in figures 22-23, such an opinion seems valid. Apparently a considerable portion of this tissue is of endodermal, and perhaps cortical, origin. Figure 23 shows the lateral root just ready to emerge from the primary. It shows the extent to which the endodermis and cortex have contributed to the building up of the tissues of the secondary roots. There is little or no solution and resorption of the primary cortex as the secondary root penetrates it. Subsequent derivation of cells of the lateral root is from its meristem, which, according to JANCZEWSKI, functions similarly to that of the primary root.

PEG

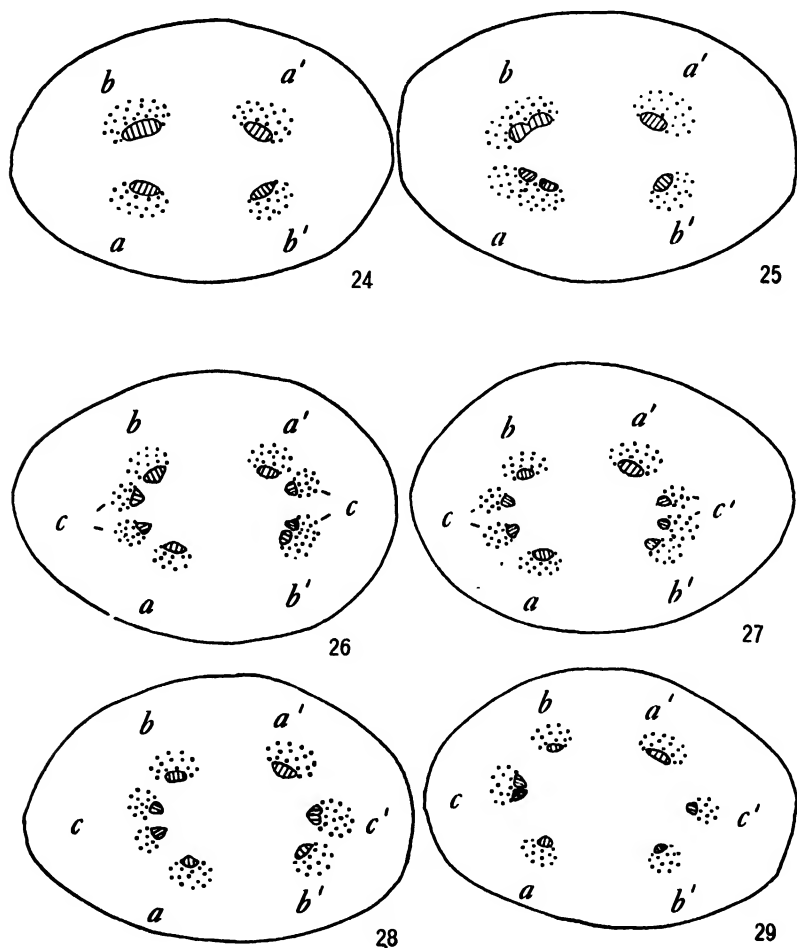
The peg is located on the axis exactly at the level of transition. Spinulose epidermal hairs, characteristic of the hypocotyl, occur on the upper face of the peg, and root hairs on its lower face. Since the peg has been adequately described by CROCKER and others (2) for other genera of the Cucurbitaceae, and since no variation from their findings was found in *Citrullus*, no detailed discussion is deemed necessary here. The most pronounced development of a peg occurs when seeds are germinated in a flat position. In such position the cortical cells composing the peg have their longest diameter in a radial plane, whereas elsewhere their longest diameter is in the vertical plane. There are also more layers of cells across the cortex at the peg than are found elsewhere in it. That it may appear at any radius or on opposite sides, in the case of seeds planted in an erect position, indicates that the peg is a localized cortical structure developed in accordance with the direction of the stimuli which initiate it.

HYPOCOTYL

As previously stated, the hypocotyl is flattened in the plane of the cotyledons. At its lowest level, about where transition is com-

pleted, there are four transition bundles. These are not uniformly spaced but are paired, with more space between the pairs than between the two bundles of a pair. This spacing of the bundles is associated with the flattening of the hypocotyl. Following transition, divergences occur in all, or at least in two, of the four bundles. Usually branches diverge from each of the two bundles which are paired toward the ends of the oval as seen in cross section. These branch bundles always come from the sides of the bundles facing each other, so that they are at the ends of the oval. They move together rapidly, and anastomose at levels varying from 1 to 5 mm. above the peg. There is some variation. Sometimes only one of each of the paired bundles branches and sometimes two of one pair may branch and only one of the other pair. In most cases both of the pairs branch. In all cases, when the process has been completed there are six bundles, now so spaced as to appear as two sets of three at opposite ends of the oval. Figures 24-29 are of transverse sections taken from successively higher levels in this zone, and show how the six bundles of the hypocotyl arise from the four transition bundles. Figure 30 is a schematic drawing made from cleared specimens showing the same features and also the xylem in transition. In many cases the bundles of the hypocotyl continue as six up to the cotyledons. In other cases additional branching may increase the number to eight. These two additional branches are also divergences from one or more of the original transition bundles. They are always from the side of the bundle opposite that from which the first branching occurred, and lie along the sides of the oval as seen in cross section. Apparently these two new bundles may come from any of the four but they are always on opposite sides of the hypocotyl, evidently providing food channels through the extensive tissues lying between the sets of bundles already present. Moreover there is no fixed level at which they diverge. Sometimes one or both may appear near the base of the hypocotyl; more commonly they appear higher up. In the region just below the cotyledons, the branch bundles lying along the flattened sides of the hypocotyl, provided such branches have formed, anastomose with one of the parent bundles between which they lie. Usually such a branch is reunited with the bundle from which it is diverged. In a few cases

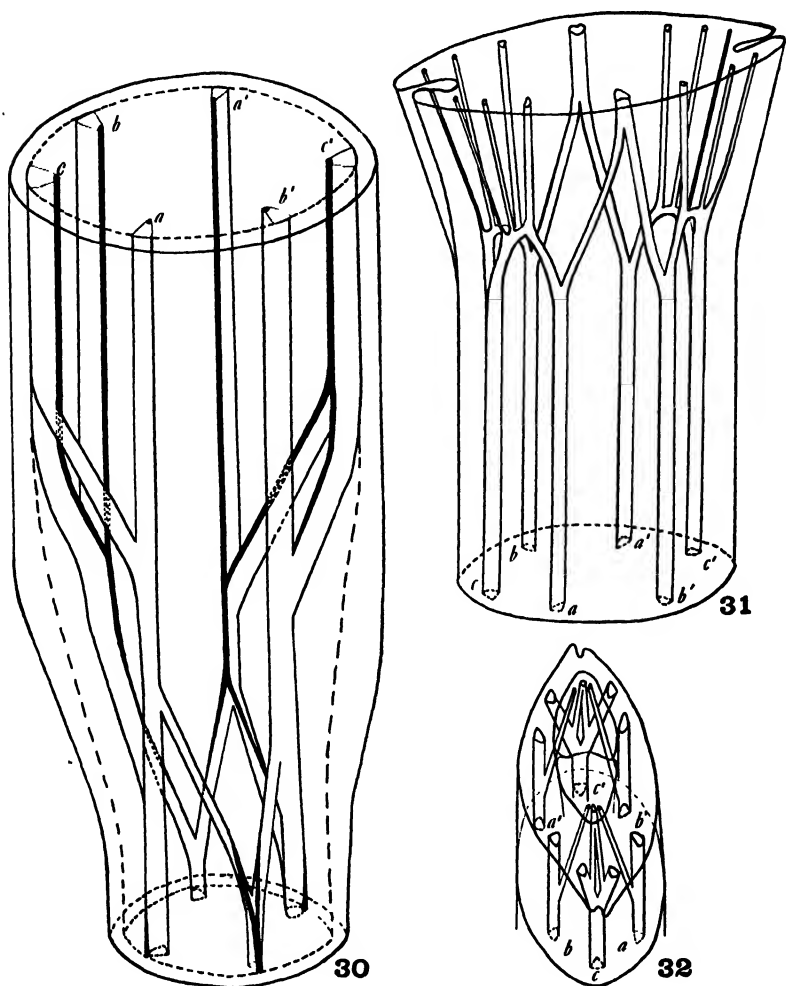
it is separated into two strands, one going to each of the two bundles between which it lies. In all cases observed this anastomosing again reduces the number to six, at or below the level at which they



FIGS. 24-29.—Diagrammatic drawings of cross sections from lower hypocotyl showing origin of six bundles of hypocotyl from four transition bundles. *a*, *a'* and *b*, *b'* are opposite transition bundles; *c* is formed of anastomosing branches from *a* and *b*, while *c'* is likewise developed from *a'* and *b'*.

enter the cotyledons. Because very often there may be no more than six bundles in the hypocotyl, and because the additional bundles, if present, are irregular as to the levels at which they occur and

always anastomose with the parent bundles, six bundles are considered the typical number, as shown in all illustrations. The



FIGS. 30-32. Schematic drawings showing general plan of vascular system: Fig. 30, root stem transition (below) and organization of six bundles of hypocotyl from four transition bundles (above). Fig. 31, manner in which hypocotyledonary bundles pass into cotyledons. Fig. 32, vascular connections to epicotyl. Bundles lettered throughout as in figs. 24-29.

bundles are bicollateral throughout the length of the hypocotyl. There is considerably more external phloem than xylem, so that it

extends far around the xylem group in each bundle. The rapid differentiation of phloem soon provides a striking preponderance of phloem. The various elements within the bundle are similar to those of the root. Figures 47-49 show these features of typical hypocotyledonary bundles. There is no interfascicular cambium. Consequently all thickening, aside from the small amount accomplished by fascicular cambium, is owing to activity of the parenchymatous tissues. This feature was reported by POTTER (9).

The epidermis is composed of greatly elongated and flattened cells. It is richly supplied with stomates.

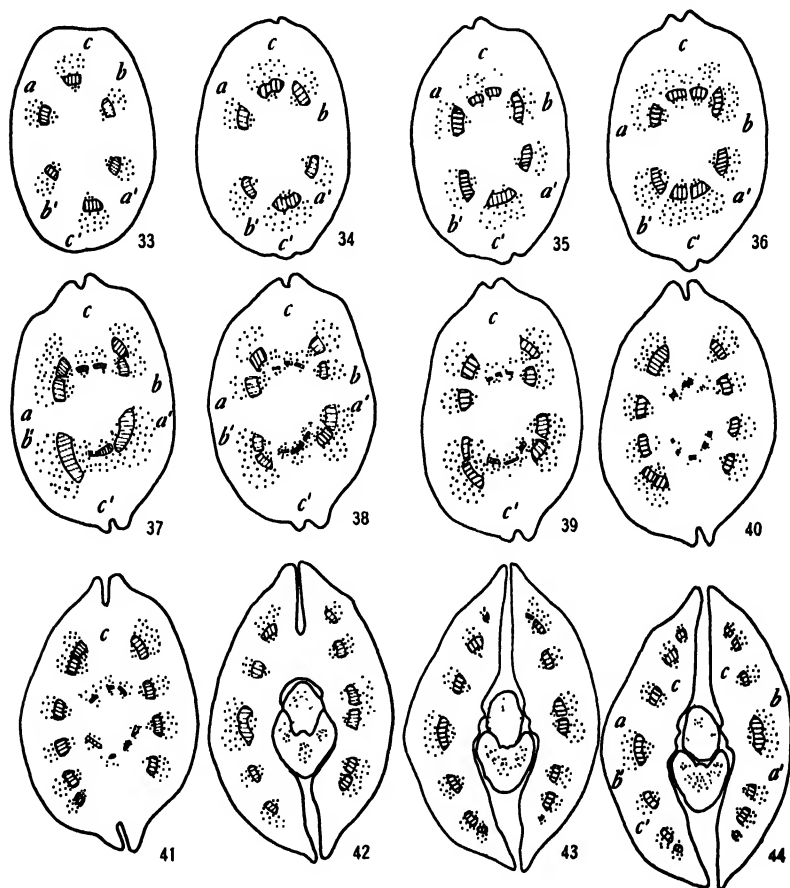
The cortex is like that of the root except that the cells become even longer, and are filled with chloroplasts to considerable depth. The endodermis and pericycle are irregular and indistinguishable as a rule. It is possible to trace the pericycle.

The central pith begins to disintegrate early throughout the length of the hypocotyl. Such disintegration is limited to the larger cells in the central region; the outer portions of the pith remain intact and are parenchymatous, especially in the region of the bundles.

COTYLEDONS

All bundles of the hypocotyl terminate in the cotyledons. There are five main bundles at the base of each cotyledon, the three centrally located ones being very prominent. In addition to the five there is a smaller branch bundle at each edge of the cotyledon. These seven form the primary framework of the whole structure. This bundle alignment is derived from the six bundle arrangement of the hypocotyl (figs. 33-44, 31). The tapering of the hypocotyl toward the top amounts almost to a constriction at the level of divergence of the cotyledons. In this region the three bundles located in each of the two ends of the oval, as seen in the cross section, are so close together as to form apparently one large bundle, in which, however, the three bundles maintain their identity. A cross section at this level (fig. 36) shows these two semicircular bundle groups with prominent rays between. At the same time the two bundles in the ends of the oval, the last formed of the six hypocotyledonary bundles, begin to divide into two separate

bundles. Immediately these, and likewise the other bundles, move outward from the semicircles toward the rays. When the separation is complete there are four bundles along each side of the oval. The two inner ones on each side are the original four transition



FIGS. 33-44. Transverse sections from upper hypocotyl showing divergence of hypocotyledonary bundles into cotyledons and vascular connections to epicotyl.

bundles, by position at least, while the two outer ones are branches from the bundles lying in the ends of the oval and formed by the anastomosing of strands from the original four. The two inner bundles of the four on each side continue to move toward each other

and finally unite to form the midribs of the two cotyledons. The outer two bundles of each cotyledonary group branch and thus increase the number to five, and this number is soon increased to seven by the smaller branches which again diverge from the outermost bundles. All the bundles of the cotyledons are loosely joined by archlike connections (fig. 31) which are produced in the apparent moving together of the hypocotyledonary bundles to form the semicircles. For the most part, these connections are composed of phloem tissue.

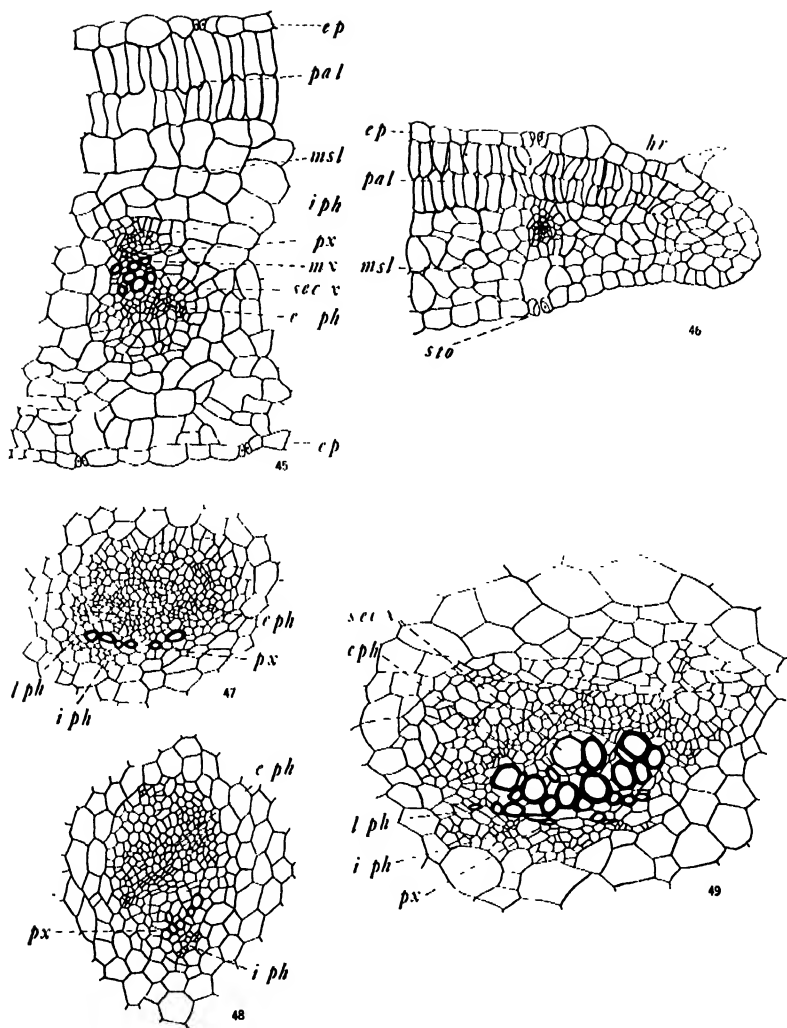
Simultaneously with establishment of their vascular framework, the cotyledons also diverge from the axis. The epidermal cells are very irregular in shape and size, presenting a mosaic-like surface view characteristic of many dicotyledonous plants. Stomata are numerous in both the lower and the upper surfaces. Their long axes lie in all directions. The hairs of the upper epidermis are simple, composed of two to five cells pyramided on a large basal cell which is surrounded by a cordon of large epidermal cells.

There are two layers of elongated cells in the palisade, which is rather loosely arranged with considerable air space, especially near the stomata. The cells of the mesophyll are more or less isodiametric, and arranged so as to provide for large air spaces in the vicinity of the stomata of the lower epidermis. They are compact around the bundles, forming a rather thick supporting sheath.

The bundles form an extensive network of veinlets through the mesophyll. They are bicollateral and undergo considerable secondary thickening. Figures 45-46 show transverse sections from the cotyledons, selected so as to show the general features described.

EPICOTYL

No extensive studies of the epicotyl were made other than to establish the nature of its vascular connections to the hypocotyl (fig. 32). There are usually six bundles in the epicotyl at the level where its first leaf diverges. These six bundles are branches from the six bundles of the hypocotyl, and have the same relative position with respect to one another and to the axis. In cross section they appear as two sets with three bundles in each set. One set of three enters the first leaf and the other set enters the second leaf. Branch-



FIGS. 45-49.—Figs. 45, 46, transverse sections of cotyledons: fig. 45 from central portion and fig. 46 at margin (*ep*, epidermis; *pal*, palisade; *msl*, mesophyll; *iph*, internal phloem; *px*, protoxylem; *mx*, metaxylem; *sec x*, secondary xylem; *e ph*, external phloem; *sto*, stoma; *hr*, epidermal hair). $\times 30$. Figs. 47-49, transverse sections of hypocotyledonary bundles showing origin of internal phloem: Fig. 47, transition bundle, at peg level, of five days old seedling (*l ph*, lateral phloem; *iph*, pith). Fig. 48 (from level just below cotyledon, same aged plant), internal phloem forming by divisions of pith cells. Fig. 49, same position as fig. 47 but of a nine days old seedling. $\times 27$.

ing of these six bundles of the epicotyl from the bundles of the hypocotyl occurs in the following manner. At or just below the level at which the cotyledons diverge from the axis, branch bundles to the epicotyl diverge from each of the four transition bundles. At this level the two remaining bundles of the hypocotyl have already branched preparatory to entering the cotyledons. Divergences to the epicotyl occur from each of these branches, and soon anastomose to form the bundles of the midribs of the first and second leaves of the epicotyl respectively. Cases occur in which these bundles of the midribs diverge as single strands from the corresponding bundles of the hypocotyl, before they branch to the cotyledons (figs. 37-41).

The divergences of the bundles to the epicotyl begin at a much earlier stage than that used for illustrative purposes. In fact their development is evident in seedlings whose hypocotyls have hardly emerged from the integuments. In such early stages, however, these nutritive connections between the axis and the epicotyl appear only as two arcs of undifferentiated, highly parenchymatous tissue lying in the zones where the actual vascular connections are to develop. Within these arcs of parenchyma again, the phloem differentiates before the xylem. The bundles are bicollateral from the beginning. It is only when the tissues of these new bundles have been well differentiated that the vascular connections to the epicotyl can clearly be determined.

When these bundles have definitely been established, a zone of tissue differentiates across the epicotyl between the two sets of bundles, and the first leaf soon diverges from the axis (figs. 32, 42-44).

Summary

1. Early stages of development of the watermelon seedling and its gross structure are described.
2. The primary root is tetrarch exarch. In transition each xylem strand is divided into two. These two divisions swing outward through arcs of 180° , and converge by pairs in front of the four phloem groups. When transition is completed there are four bundles composed of the four original phloem groups intact and of xylem which comes in approximately equal amounts from two adjoining

groups of xylem of the original alignment. The transition is rapid and is completed at the level of the peg.

3. There is one common histogen in the primary root; all primary tissues are derived from a common meristematic region.

4. The endodermis and inner cortical layers, as well as the pericycle, of the primary root contribute to the building up of the tissues of the secondary roots.

5. There are usually six bundles in the hypocotyl: the four transition bundles and two others which are anastomosed branches from the transition bundles. All these bundles diverge into the cotyledons.

6. For a considerable period of time the cotyledons serve as photosynthetic organs.

7. The epicotyl develops slowly at first, because its vascular connections with the axis are, in the main, differentiated after germination.

8. The bicollateral bundles extend from the level at which transition is completed upward through the hypocotyl into the cotyledons and into the epicotyl. The internal phloem is derived from the parenchyma which lies adjacent to the inner faces of the bundles. It does not extend into the roots, but is connected laterally with the external phloem in the transition zone.

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EMBRYOLOGY OF PISUM SATIVUM¹

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(WITH THIRTY-ONE FIGURES)

Introduction

Little information concerning the embryology of *Pisum sativum* L. or of any other member of the Viciae tribe of the Leguminosae is to be found in the literature. Perhaps the earliest reference is by TULASNE (9), who described stages in the development of the embryo of *Lathyrus aphaca* L. He reported the presence of a suspensor of peculiar type, consisting of two long filiform basal cells and two shorter but larger cells closely adjacent to the embryo. HOFMEISTER (6) found that the embryo of *Lathyrus odoratus* L. is pushed to the mid-region of the embryo sac as a result of elongation of the suspensor. STRASBURGER (8) called attention to the multinucleate condition of the suspensor cells in *Orobus*. The most extensive study of the embryology of members of this tribe is that of GUIGNARD (5), who examined twenty-three species, including eight of *Orobus* (*Lathyrus*), six of *Vicia*, two of *Pisum*, and two of *Ervum* (*Vicia*). He described in detail the development of the embryo sac and embryo of *Orobus angustifolius* L. (*Lathyrus canescens* Gren. and Godr.), and indicated that the same type of development is characteristic of other members of the tribe. MARTIN (7) found a similar type of embryo development in *Vicia americana* Muhl.

Material and methods

The material for this investigation was collected from plants growing in the greenhouses and field plots of the Department of Genetics, University of Wisconsin, during the spring and summer of 1937. Two commercial varieties (Little Marvel and Asgrow Pride) of the

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common garden pea (*Pisum sativum*) were used. Ovaries from late buds and open flowers and pods of various ages were immersed in Carnoy's alcohol-acetic-chloroform mixture for 2-5 minutes, and then transferred to Karpechenko's modification of Navashin's fluid. The older pods were opened and the young seeds removed and similarly treated. After imbedding in paraffin, sections were cut from 12 to 20 μ in thickness (depending upon the age of the material), mounted serially, and stained in dilute Delafield's haematoxylin. No significant differences were found between the two varieties studied. The description which follows is based chiefly upon Asgrow Pride.

Observations

POLLINATION AND FERTILIZATION.—Each ovary contains from eight to ten campylotropous ovules arranged alternately along the ventral suture. The curvature of each developing ovule is toward the style. At maturity the micropyle is thus brought into close proximity to the ventral suture, the opening of the micropyle facing the basal end of the ovary.

The mature embryo sac just before fertilization is an elongate, seven-celled, eight-nucleate structure. The curvature of the ovule has caused the embryo sac to be bent at an obtuse angle in the mid-region. The three irregularly shaped antipodal cells at the chalazal end of the sac are very small and disintegrate shortly after fertilization.

The egg apparatus consists of three pear-shaped cells. The egg, which is much larger than either synergid, contains a large nucleus imbedded in the dense cytoplasm in its basal region and a large vacuole at the micropylar end. A large vacuole occupies the enlarged basal portion of each synergid, and a series of elongate vacuoles or canals extends forward from this region toward the apex of the cell. The nucleus lies in the dense cytoplasm near the mid-portion of the cell, just above the basal vacuole.

The two polar nuclei are closely appressed. They lie near the bend of the embryo sac in a mass of denser cytoplasm which extends from the egg apparatus toward the chalaza.

Pollination takes place approximately 24 hours before the open flower stage is reached. The standard at this time is greenish white

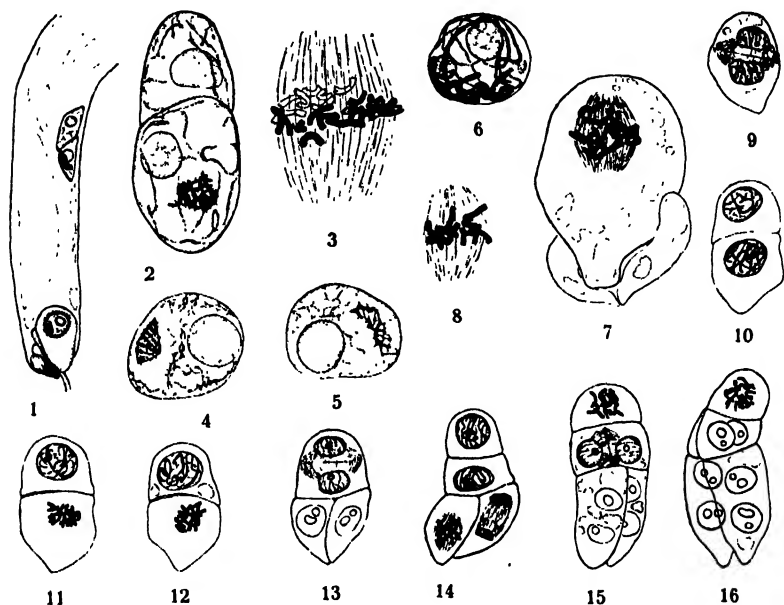
in color; the wings more or less tightly inclose the keel. Fertilization has taken place, and either the zygote is in the process of division or a two-celled proembryo is present at the time the flower is fully expanded. The pollen tubes grow along the ventral suture of the ovary, and when they reach the micropyle of the ovule one or more attempt to enter it. One pollen tube grows into the micropyle and enters the embryo sac between the apices of the synergids and egg (fig. 1). Although in some instances many pollen tubes were observed at the entrance of the micropyle, no cases were observed in which more than one had entered the embryo sac. The synergids are not broken down by the pollen tube as in *Phaseolus vulgaris* (10), but persist for a short time after fertilization, as in *Melilotus* and *Medicago* (2, 3).

The two male gametes are discharged from the pollen tube into the embryo sac in the vicinity of the egg. Each male gamete, as found in the pollen tube, is surrounded by a definite layer of cytoplasm (4). Whether the cytoplasmic sheaths are shed when the gametes leave the pollen tube or after they have entered the embryo sac was not determined. One male gamete nucleus becomes closely appressed to the egg nucleus in the process of fertilization; the other becomes appressed to one of the polar nuclei (fig. 1). Each male nucleus is somewhat elongated with a nucleolus near one end as in *Medicago* (3) and not rounded as in *Phaseolus* (1).

The small densely staining male gamete nucleus, which is appressed to one of the polar nuclei, increases in size, the nuclear membranes disappear along the surface of contact, and the chromatic network of the male nucleus spreads out but never as extensively as does that of either polar nucleus (fig. 2). The three nuclei of this group divide simultaneously, and the groups of chromosomes are brought on to a common spindle (fig. 3). The three chromosome groups may be identified in figures prior to and including the equatorial plate stage.

The second male nucleus becomes closely associated with the egg nucleus, and the nuclear membranes disappear in the region of contact (fig. 4). The chromatic network of the male nucleus spreads out for a time (fig. 5), and then condenses in advance of that of the egg nucleus. As a result, short well developed chromosomes appear

in one portion of the fusion nucleus, whereas the remainder of the nucleus contains elongated chromatic threads in an early prophase condition (fig. 6). The nucleolus brought in by the male nucleus



FIGS. 1-16.—Fig. 1, micropylar portion of embryo sac showing double fertilization. Fig. 2, male gamete nucleus fusing with polar nuclei. Fig. 3, first division of primary endosperm nucleus showing three separate groups of chromosomes. Fig. 4, early stage in process of fusion of male gamete nucleus with egg nucleus. Fig. 5, somewhat later stage; limits of male gamete nucleus still clearly defined. Fig. 6, zygote nucleus in prophase; chromosomes from egg nucleus less condensed than those from male nucleus. Fig. 7, egg apparatus; zygote with lateral view of equatorial plate; chromosomes at right side of spindle on plate, those on left side being drawn to plate. Fig. 8, lateral view of zygote equatorial plate showing two groups of chromosomes. Fig. 9, binucleate proembryo showing cell plate formation. Fig. 10, two-celled proembryo. Figs. 11, 12, two-celled proembryos; nuclei of basal cells in division; nuclei of apical cells in prophase. Fig. 13, three-celled proembryo; cell plate formation in apical cell. Fig. 14, four-celled proembryo; nuclei of two basal cells in division. Fig. 15, same; basal cells binucleate; cell plate formation in middle cell; nucleus of apical cell dividing. Fig. 16, five-celled proembryo; two binucleate basal cells, two uninucleate middle cells; nucleus of apical cell dividing. Fig. 1, $\times 185$; figs. 2-8, $\times 880$; figs. 9-16, $\times 450$.

enlarges to some extent but never approaches the size of the nucleolus of the egg.

Two distinct groups of chromosomes can be recognized on the

mitotic spindle in the zygote (figs. 7, 8). The chromosomes on the right side of the spindle shown in figure 7 are in a plane above those on the left side. In the plate shown in figure 8, likewise, there is a definite plane of separation between the two groups. The details of gametic fusion and the division of the zygote nucleus are very similar to those previously described for *Medicago* (3).

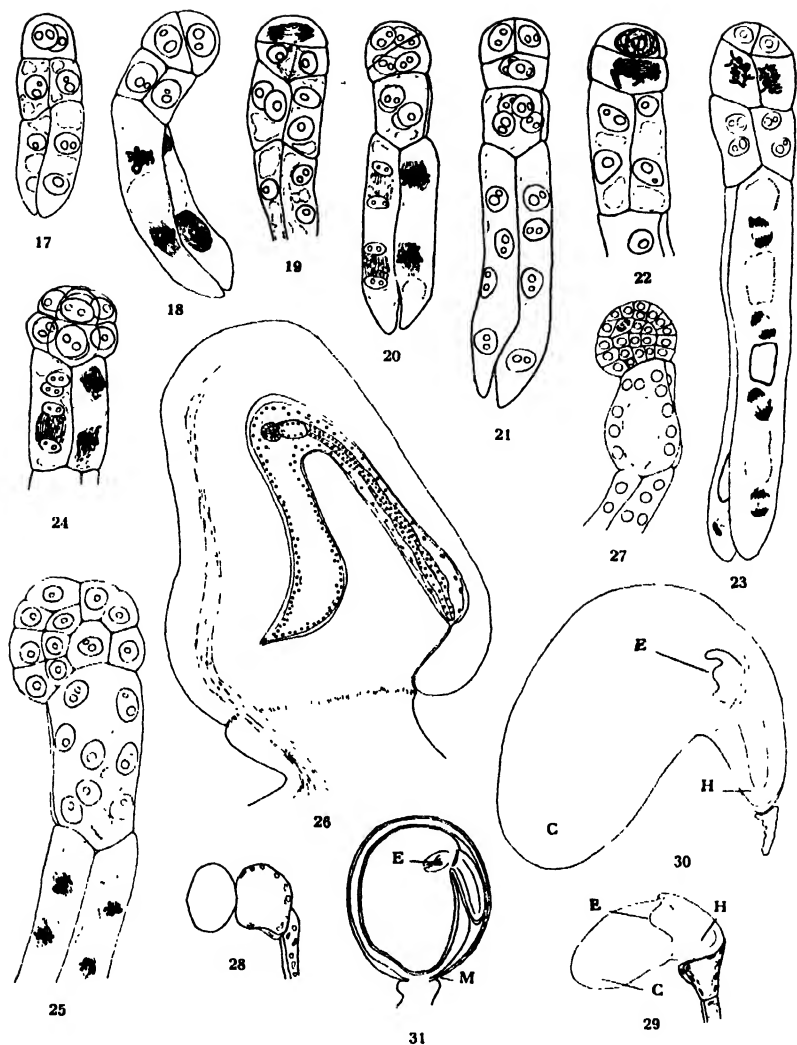
ENDOSPERM.—One of the two nuclei formed as a result of the division of the triple fusion nucleus migrates toward the chalazal end of the embryo sac and the other to a position near the zygote. Shortly thereafter a second division occurs and the four resulting nuclei take peripheral positions in the dense cytoplasm surrounding the large central vacuole. The endosperm possesses usually two, sometimes four, nuclei before completion of the division of the zygote nucleus. This is similar to the condition found in *Melilotus* and *Medicago* (2, 3). GUIGNARD (5) found that the zygote of *Orobis angustifolius* divides prior to the first division of the primary endosperm nucleus, and MARTIN (7) found that the divisions of zygote and primary endosperm nuclei are simultaneous in *Vicia americana*. No instances have been found in the material of *Pisum* thus far examined of a division of the zygote nucleus before division of the primary endosperm nucleus.

Divisions of endosperm nuclei continue in rapid sequence. The early divisions are simultaneous, but at later stages of development this may not be the case. The dividing nuclei in any particular area of the endosperm, however, are in approximately the same stage of mitosis. Thus the divisions of those nuclei in the region of the embryo may be at metaphase and those in the chalazal region in a prophase stage.

The endosperm is multinucleate at stages such as that shown in figure 26. Nuclei are present in greater numbers in the dense cytoplasm immediately surrounding the embryo than elsewhere in the endosperm. With the exception of the region immediately adjacent to the hypocotyl and extending from there to the micropyle, the endosperm remains multinucleate throughout its development. Cell formation is initiated in the neighborhood of the hypocotyl and of the basal portions of the cotyledons in ovules in which the embryo has reached the stage shown in figure 29. It continues toward the

micropyle so that all the endosperm in this region becomes cellular. According to GUIGNARD, cell formation is totally absent in the endosperm of members of the Viciaceae. The endosperm of *Pisum* is resorbed in the course of embryo development, and there is little if any evidence of it in the mature seed.

EMBRYO.—The zygote divides transversely (fig. 9) to form a two-celled proembryo, the two cells being approximately equal in size (fig. 10). The basal cell (that is, the one nearer the micropyle) now divides longitudinally to form two suspensor cells (figs. 11–13). Shortly thereafter the apical cell divides transversely, forming an apical embryo mother cell and a middle cell (figs. 13, 14) which in the course of development is to form a bulbous middle piece. The nuclei of the two basal suspensor cells divide (fig. 14), and, cytokinesis not following, binucleate cells are formed (fig. 15). Next occurs a longitudinal division of the middle cell, the plane of division being at right angles to that which occurred to produce the two basal cells. Shortly thereafter the nucleus of the apical embryo mother cell divides, the long axis of the division spindle being at right angles to the plane of division of the original middle cell (figs. 15–17). The plane of cytokinesis in the apical cell is at an oblique angle (fig. 18). The basal cells have been elongating during this period of development, and their nuclei as well as those of the middle cells divide, producing respectively four-nucleate and binucleate cells. The divisions in the basal cells in some instances precede those in the middle cells (fig. 18); in other cases they follow (fig. 20). The two cells of the embryo proper divide longitudinally, the planes of division being at right angles to each other, to form a four-celled embryo (figs. 19–21). Each of these cells divides again (figs. 22, 23), producing an eight-celled embryo consisting of two layers of four cells each (fig. 24). Nuclear divisions but no cell divisions occur in the suspensor cells, so that each of the basal cells has eight nuclei and each of the middle cells has four nuclei by the time an eight-celled embryo is formed (figs. 23, 24). The cells of the embryo continue to multiply, and an undifferentiated spherical mass of cells is formed (fig. 27). The suspensor in the meantime continues to develop (fig. 25) and reaches its greatest size at a stage such as shown in figure 26. The two basal cells, which have elongated and now



FIGS. 17-31. -Fig. 17, two-celled embryo, four-celled suspensor; $\times 450$. Fig. 18, same; two nuclei of each basal cell dividing; $\times 450$. Fig. 19, embryo showing divisions leading to four-celled stage; $\times 450$. Figs. 20, 21, four-celled embryo and suspensor; basal cells of suspensor have four nuclei; middle cells binucleate; $\times 450$. Figs. 22-24, divisions in embryo leading to eight-celled stage; fig. 23 shows dividing nuclei in basal cells and fig. 24 similar divisions in middle cells; $\times 450$. Fig. 25, somewhat later stage; middle cells eight-nucleate; nuclei of basal cells dividing; $\times 450$. Fig. 26, longitudinal section of young seed showing greatest development of suspensor; elongating basal cells have pushed middle piece and embryo to region of bend of ovule; $\times 45$. Fig. 27, embryo at slightly later stage; $\times 185$. Fig. 28, embryo somewhat broadened and flattened; nuclei of suspensor cells in early stages of disintegration; $\times 45$. Fig. 29, early differentiation of parts of embryo: C, cotyledon; E, epicotyl; H, hypocotyl; $\times 23$. Fig. 30, older embryo showing bend in region of epicotyl; $\times 23$. Fig. 31, longitudinal section through mature seed showing relation of parts: M, micropyle; $\times 3$.

possess sixty-four nuclei each, have pushed the embryo and the middle cells into the multinucleate endosperm so that they lie in the region of the bend of the ovule. The middle cells have meanwhile grown to form a bulbous middle piece between the basal cells and the embryo. Each of the middle cells has thirty-two nuclei, imbedded in the peripheral layer of dense cytoplasm. A large vacuole occupies the central region of each middle cell. The bulbous middle piece is at this stage fully as large as, if not larger than, the embryo proper. The nuclei of the cells of the suspensor do not divide further. The cells, especially those of the middle piece, may grow to some extent (fig. 28).

With continued development, the embryo broadens and flattens, and that portion adjacent to the middle piece becomes rounded (fig. 28). Certain cell groups on opposite sides of the periphery of the apex, at the center of the apex, and at the base of the embryo become actively meristematic (fig. 29) and the cotyledons (*C*), epicotyl (*E*), and hypocotyl (*H*) are initiated. In figure 29 one cotyledon is removed to show the epicotyl. The nuclei of the suspensor show early signs of disintegration at stages such as that of figure 28, and there is definite distortion of these cells shortly thereafter (fig. 29). The suspensor from this time on rapidly disintegrates, and by the time the embryo has reached the stage of development shown in figure 30 it is in an advanced stage of disorganization. No definite remains of a suspensor could be identified in the mature seed.

The cotyledons and hypocotyl elongate, the former toward the chalaza and the latter toward the micropyle. The embryo becomes curved in the region of the epicotyl to conform to the shape of the ovule (fig. 30). The cotyledons develop at the expense of the endosperm, becoming large rounded structures which occupy most of the space in the chalazal end of the ovule. They act as storage structures, and the endosperm is completely assimilated. The hypocotyl likewise elongates but to a lesser extent, so that at maturity its tip lies approximately halfway between the micropyle and the epicotyl. The longitudinal axes of the hypocotyl and of the cotyledons are approximately parallel in the mature seed. Primordia of the first secondary leaves develop just below the apical meristematic tip of the epicotyl. Figure 31 represents a longitudinal section

through a mature seed. The relationships existing between the various parts just described are shown in this figure. The course of embryo development in *Pisum* is essentially similar to that described by GUIGNARD for *Orobis angustifolius*.

Summary

1. Pollination takes place in *Pisum sativum* between 24 and 36 hours before the open flower stage. Fertilization has occurred and division of the zygote is under way by the time the flower is fully opened.

2. The pollen tube enters the embryo sac between the synergids, neither of which disintegrates until later.

3. One male gamete nucleus fuses with the two polar nuclei. In the division of the primary endosperm nucleus three separate groups of chromosomes are recognizable on the equatorial plate.

4. The primary endosperm nucleus divides before the zygote nucleus. Free nuclear division occurs in the endosperm in the early stages of development. Cell formation later takes place in the micropylar portion of the endosperm, the chalazal portion remaining multinucleate. The endosperm is resorbed in the course of embryo development.

5. During the division of the zygote nucleus, the chromosomes from the two gamete nuclei remain in more or less distinct groups until the equatorial plate stage.

6. The zygote divides transversely to form a two-celled proembryo. The basal (micropylar) cell then divides longitudinally to form two suspensor cells, and the apical cell divides transversely to form an apical embryo mother cell and a middle cell. The middle cell divides longitudinally to form a two-celled middle piece. The nuclei of the two basal and two middle cells divide to form multinucleate suspensor cells having respectively sixty-four and thirty-two nuclei each.

7. The embryo develops in a typical manner from the apical embryo mother cell.

8. The two multinucleate basal cells elongate and push the embryo and middle piece to the region of the bend of the campylo-tropous ovule.

9. The suspensor cells disintegrate shortly after differentiation of the cotyledons, epicotyl, and hypocotyl.

10. The cotyledons act as storage organs in the mature seed, the endosperm being completely assimilated in the course of embryo development.

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AUXIN DISTRIBUTION IN SEEDLINGS AND ITS BEARING ON THE PROBLEM OF BUD INHIBITION

J. VAN OVERBEEK

(WITH TEN FIGURES)

Introduction

The question of auxin distribution throughout the plant is obviously of importance for the understanding of growth. Of the plants commonly used for the study of growth phenomena, the *Avena* seedling is the only one that has been satisfactorily analyzed in this respect (14).

Recently *Zea* and *Pisum* have become important laboratory plants, which has made it necessary to know more about their auxin distribution. Maize is used in these laboratories for the study of the physiology of dwarf plants (19, 24) and for investigations on ageotropic corn (21). The pea is used for the pea test (32, 33), for root formation (31), for grafting (29), bud inhibition (12, 16, 17), and various other experiments.

In this paper an attempt is made (1) to analyze the auxin distribution throughout etiolated maize and pea seedlings; (2) to see how far the distribution of growth in maize seedlings can be explained on the basis of auxin distribution; (3) to study, in pea seedlings, the changes in auxin content and distribution resulting from removal of the terminal bud and subsequent development of a lateral bud—in this connection it was also of interest to know how the auxin distribution is changed in decapitated pea plants to which auxin was applied; (4) to see how the data obtained under (3) fit in with the existing theories on bud inhibition. Part I of this paper deals with (1) and (2); part II with the problem of bud inhibition.

Method

THIMANN (14) was the first to study auxin distribution in plants. He found that in *Avena* seedlings the auxin content decreased from the tip to the base of the coleoptile. This was determined by ex-

tracting auxin with chloroform from crushed and acidified plant material. Other extraction methods have been mentioned in a previous paper (23). When tried on maize, none of the methods so far described proved to be satisfactory for the extraction of auxin. The acid commonly used by all methods especially decreased the yield. Table 1 gives the result of one experiment in which the extraction

TABLE 1

EFFECT OF ACID ON EXTRACTION. AUXIN DISTRIBUTION IN OAT SEEDLINGS GROWN IN WATER CULTURE AND IN SAND (NOS. 70802, 70928)

EXTRACTION METHOD	PLANT MATERIAL	AMOUNT OF AUXIN EXTRACTED IN DEGREES OF CURVATURE IN AVENA TEST		
Crushed and boiled with 50 cc. ether and 1 drop of 1 N acetic acid for 1 hour	14 corn seedlings	With acid	5.7	
		Acid omitted	13.3	
Not crushed, left for 15 hours in 50 cc. ether and 0.5 cc. of 0.1 N acetic acid	50 oat seedlings	Amount of auxin per cm.		
		Roots in water		Roots in sand
		Acid	No acid	No acid
	Coleoptile (plus leaf)			
	Upper third	7.7	7.7	7.1
	Middle third	2.9	2.7	3.0
	Lower third	8.8	19.9	15.5
	Mesocotyl
	Upper third			5.4
	Middle third	14.0*	11.8*	9.3
Lower third	10.9	

* Since the entire mesocotyl of watergrown plants was only 4 mm., the actual figures obtained in the assay were 5.6 and 4.7.

with and without acid was compared. The explanation of this destructive effect of acid may be found in the high auxin-b content of corn plants, which is unstable with acid, as was found by KÖGL, ERXLEBEN, and HAAGEN SMIT (4).

In many of the methods the material is crushed, but this procedure was not followed because a prolonged extraction was found to extract the auxin sufficiently. Furthermore, crushing involves much labor when large amounts of material or many samples are used.

Ether was employed for the extraction in this work because it is easier to separate from plant material and water than is chloroform, and its low boiling point makes it easy to concentrate the extracts by distilling the ether off. The entire procedure is as follows.

a. Ether was purified by shaking with FeSO_4 , CaO , and some water (amounts used are of minor importance), followed by distillation to recover the ether. Purification is very important and was

TABLE 2
WATER CONTENT AND LENGTH OF SEEDLINGS
(NOS. 71012, 14, 22)

PART OF PLANT ANALYZED	TOTAL LENGTH (CM.)	AMOUNT OF WATER PER PLANT IN MG. PER CM. (IF BUDS, IN MG. PER ENTIRE BUD)
Corn		
Coleoptile..	2 1	20.5 (92.0%)
Mesocotyl..	6.4	34.5 (93.5)
Leaf.....	2 0	7 1 (85.5)
Oats.....		
Coleoptile.	2 8	7 7 (93.9)
Mesocotyl	0 3	16.3 (91.8)
Leaf....	2 7	3 0 (87.5)
Peas		
Terminal bud.	..	30 1.4 (85.4)
Upper internode ..	4.9	24.5 (93.5)
Upper lateral bud	2 20 (88.1)
Middle internode.....	4 3	31 2 (95.0)
Lower lateral bud	1 65 (88.8)
Lower internode.....	4 2	35 7 (92.3)

done immediately before an extraction was made. The fact that a sample of ether gives a negative benzidine test (26), a test which is considered extremely sensitive for detecting peroxides, does not mean that it is pure enough for use in extracting auxins. Such a sample may completely destroy an indoleacetic acid solution when shaken with it.

b. The plant material was weighed, or simply measured if the weight and water content per unit length of a certain batch of plants was known (table 2), cut into the parts to be analyzed, and placed (uncrushed and not acidified) in Erlenmeyer flasks. Then 25 to 50 cc. of ether was added per gram of plant material. The total amount

was never less than 50 cc. This was left in the refrigerator for about 20 hours at 4° C. If for some reason the material could not be tested after this period of time, it was found advisable to evaporate the extract to dryness (see c and d) and store it in the dry condition rather than leaving it in the ether.

c. The ether was then poured off and the residual plant material rinsed with pure ether. Next the ether extract was concentrated by distilling the ether off (water bath) until about 1 to 2 cc. was left.

d. The residue was taken up in a pipette and dropped carefully on the bottom of a small (5 cc.) vial suspended in a beaker of boiling water. Container and pipette were rinsed with a few cc. of pure ether, which also was carefully evaporated into the vial. Thus the extract was evaporated to complete dryness and collected on the bottom of the vial.

e. A known amount of agar (formerly 3 per cent was used, later 1.5 per cent) was next pipetted on the dry extract. As a minimum amount, 0.5 cc. was used. This was stirred and shaken (the vial still in boiling water) to secure a thorough mixing of auxin and agar. After the vial was removed from the boiling water, it was allowed to stand at room temperature for about three hours before the agar was poured into blocks for the *Avena* test. This procedure proved to be necessary in order to obtain a uniform distribution of the auxin in the agar.

f. The agar was melted again in boiling water and poured into a rectangular brass mold ($8 \times 10.5 \times 1.7$ mm.), which was cooled by ice for a rapid gelification of the agar. Next this agar plate was divided into twelve equal blocks, which were ready now to be put on decapitated oat seedlings in order to determine their auxin concentration.

g. The *Avena* test employed has been described by WENT and THIMANN (33). A control with a known amount of indoleacetic acid was run at the same time in order to calculate the obtained values of the extract in gammas (0.001 mg.) indoleacetic acid.

h. If the water content had to be determined, the length of the stems, etc., was measured first; then the fresh weight was determined; next the material was left for twenty-four hours in an electric drying oven at 100° C. and the water content determined.

i. The concentration of the auxin in the plant may be calculated from:

$$\frac{C \times I_r \times V_a}{W} \text{ gammas indoleacetic acid equivalents}$$

per 1000 gm. water contained in the plant. C is the curvature obtained in the *Avena* test when the agar blocks (see f) are analyzed. I_r is the concentration of indoleacetic acid (gammas per liter) required to give an increase in curvature of 1° in the *Avena* test. This value, of course, is obtained by calculation from auxin concentrations giving a curvature in the range between 5 and 15° . V_a is the volume of the agar in which the residue is taken up (in cc.). W is the water content (in grams) of the plant material extracted.

It should be noted that in this way the auxin content of the plant material is expressed in terms of indoleacetic acid. In the higher plants auxin of the auxin-a type rather than indoleacetic acid may be expected to be present. The activity of auxin-a, according to KÖGL and co-workers, is about twice that of indoleacetic acid. Throughout this paper most amounts of auxin are expressed in indoleacetic acid equivalents. This provides a unit which is independent of the sensitivity of the test plants, and even of the kind of test plant used, thus making comparable, for instance, values obtained by the regular *Avena* test with such obtained by a *Cephalaria* test carried out in the light (9).

Part I: Auxin concentrations in *Zea* and *Avena*

DISTRIBUTION OF GROWTH AND OF AUXIN IN *ZEa* SEEDLINGS

Corn seedlings were grown in moist sand in a physiological dark-room at 24° C. and 90 per cent humidity. The distribution of the growth rates of these seedlings was measured by marking each seedling into 1 mm. segments. This was done by stamping thin parallel ink lines on the coleoptile and mesocotyl with a device described by VAN OVERBEEK and WENT (25). After a certain period of time ranging from two hours (fig. 3) to twelve hours (fig. 1), the increase in length of the segments was determined. The growth rate of intact plants (curve *i* of fig. 1) increases gradually from the tip to the base of the coleoptile. In the apical region of the mesocotyl,

which is called first internode by AVERY and co-workers (2), the growth rate has its highest value. The lower part of the mesocotyl had stopped growing at the time the measurements were made.

For explaining the growth distribution in etiolated seedlings of grasses, WENT (27) devised the so-called two-factor scheme. This scheme (33, p. 78) is based on the interaction of two factors both necessary for growth. Auxin, one of the factors, is produced at the tip of the plant; it is transported downward and is used up on its

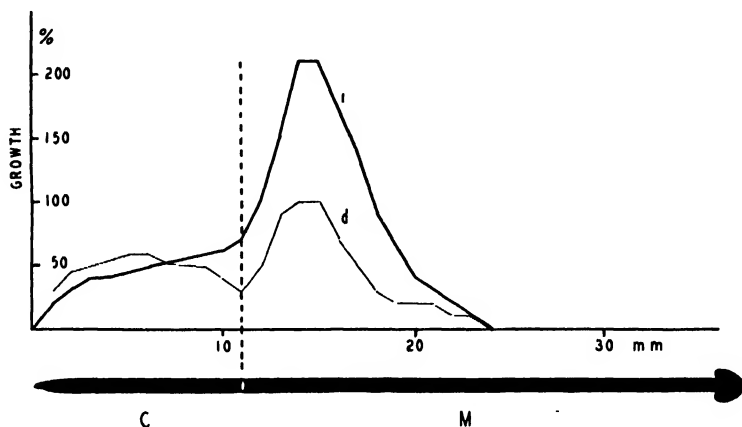


FIG. 1.—Distribution of growth in 4-days-old maize seedlings. Ordinate: percentage growth in 1 mm. segments per 12 hours. Abscissa: location of these segments originally 1 mm. long. *i*, intact plants; *d*, plants from which coleoptile tip was removed 12 hours before final measurements. Average values of 10 uniform plants. No. 60901.

way down. The other factor (or complex of factors) is called the “food factor”; it is supplied by the seed and transported in an upward direction. The food factor too is supposed to be used up while transported. From this consideration it is clear that the growth of the apical regions of the coleoptile is not limited by auxin but by the food factor. On the other hand, the growth of the basal regions of the coleoptile and of the entire mesocotyl is limited by the available amounts of auxin in these regions.

In this connection it was shown by VAN OVERBEEK (20) that corn seedlings in which the auxin production was reduced by a heat treatment showed a slight reduction in length in the coleoptile, but the mesocotyl of the treated plants had less than half the length of that

of the controls. If the coleoptile tip of the treated plants was supplied with additional auxin, then the mesocotyl of the treated plants reached the same length as the controls. Similarly it was shown (19, 24) that plants in which the auxin is destroyed more rapidly than is normally the case have a mesocotyl which is much shorter than that of normal plants. Such plants are dwarfs. In the dwarf race *nana*, which is shown to have an excessive auxin destruction, the seedlings have coleoptiles which are only a little shorter than those of comparable normal plants; on the other hand, the mesocotyls are several times longer in the normal plants than in the dwarfs. Growth of the mesocotyl does not depend entirely on elongation but also to some extent on cell division (1).

How well the two-factor scheme fits in with observations may again be seen from figure 1. Here the auxin production of one set of plants was reduced by removing the upper portion of the coleoptile (about 2 mm.). For a few hours after the decapitation no auxin is produced, but later the auxin production is resumed in the top regions of the coleoptile, but at a reduced rate (33). Curve *d* of figure 1 represents the growth curve of such decapitated plants. The growth rate of the upper regions of the coleoptile is slightly increased over that of the intact plants. In the lower regions and in the mesocotyl the growth rate of the decapitated plants is less than that of the intact ones. According to the two-factor scheme this is to be explained as follows. The lower growth rate at the basal regions is due to the reduced auxin production of the decapitated plants because auxin limits the growth of these regions. The increased growth rate of the upper regions of the coleoptile of the decapitated plants is due to the larger supply of food factor in these regions. The basal regions of the decapitated plants have a smaller growth rate than those of the intact ones, hence less food factor is used up in the basal regions of the decapitated plants and consequently more of it will be available for the upper regions of the decapitated plants than for these regions of the intact plants.

The etiolated corn seedling shows a peculiar growth maximum in the upper portion of the mesocotyl, as is shown in figures 1 and 3. For explaining this growth maximum two possibilities may be considered.

1. The maximum may be due to an increase in auxin production in or just above the fast growing region. However, determinations of the auxin content in the various parts of the plant show that the auxin concentration is lowest in the upper portion of the mesocotyl (figs. 3, 4). Any increase in production in this upper portion necessarily must be reflected in an increased auxin content in these regions. Hence this possibility can be safely dismissed.

2. The maximum may be due to an increased sensitivity of the upper portion of the mesocotyl. Evidence that this region is very sensitive to auxin is obtained from figure 2. In this figure plants are shown to which a long narrow strip of auxin paste (lanolin in which indoleacetic acid was dissolved) was smeared unilaterally from the coleoptile tip to the mesocotyl base. After about half an hour the corn seedlings showed the following response: upper part of coleoptile remained straight; basal part of coleoptile slightly bent; apical part of mesocotyl strongly curved; basal part of mesocotyl straight.

Other evidence in the same direction is found in figure 1. In the basal region of the coleoptile the intact plants have elongated 70 per cent over the initial length. The decapitated plants in the same period of time have grown 30 per cent in this region. The ratio of the growth rate intact plants/decapitated plants is 2.3 for this basal region of the coleoptile. The intact plants show a maximal mesocotyl growth of 210 per cent over the initial length. The decapitated plants show a maximal mesocotyl growth of 100 per cent. Hence for the upper portion of the mesocotyl the ratio of the growth rate intact plants/decapitated ones is 2.1. This clearly indicates that the growth reduction due to decapitation is the same in the basal part of the coleoptile as in the upper portion of the mesocotyl. If it were not for the great sensitivity in the upper portion of the mesocotyl, the growth distribution curve would look very much like the one found for *Avena* seedlings grown under ordinary darkroom conditions. Such a curve does not have a maximum as pronounced as the one shown in the curves of figure 1 (see 33, p. 82). WENT (27), studying the mesocotyl growth of *Avena*, also came to the conclusion that in this plant the mesocotyl must have a great sensitivity.

The auxin in seedlings of grasses is produced in the tip of the coleoptile; consequently a high auxin concentration is found in the

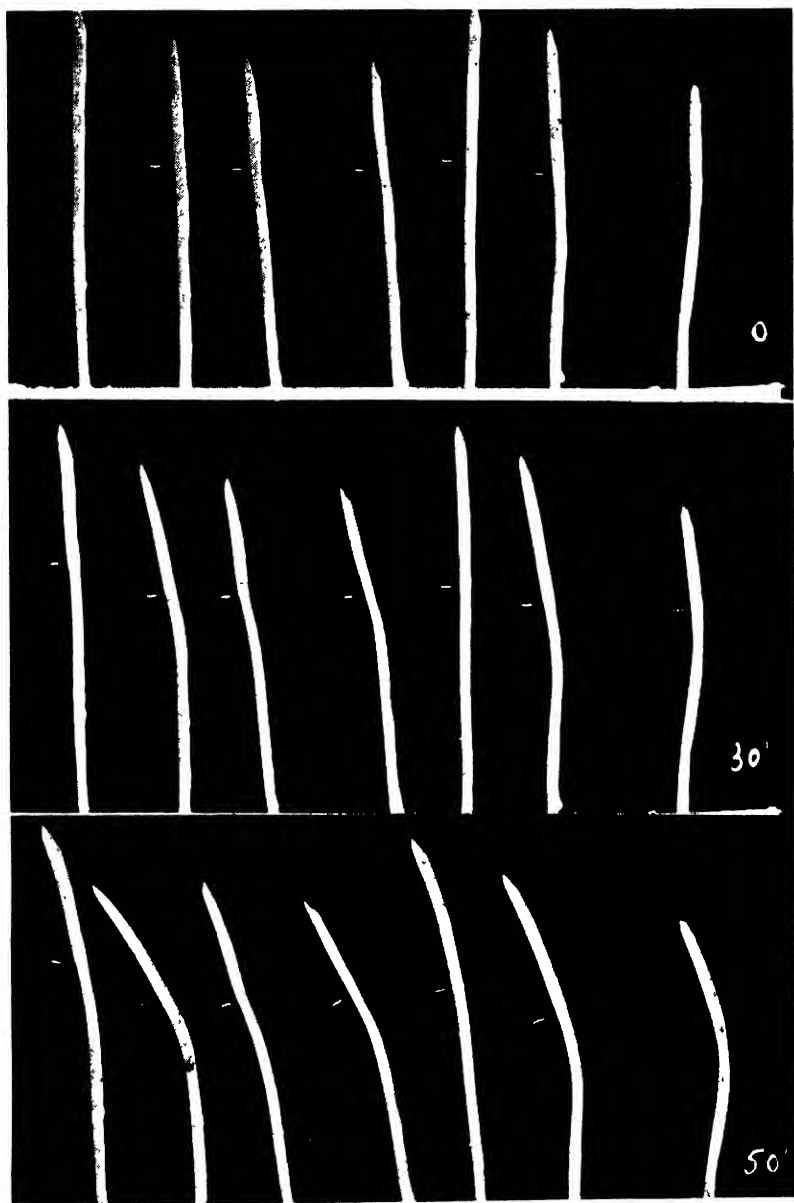


FIG. 2.—Auxin paste (0.0036% indoleacetic acid) smeared along whole right side of 4-days-old corn seedlings. Upper, immediately after auxin application; other rows, 30 and 50 minutes after auxin application. White lines at left side of each plant indicate location of coleoptilar node. No. 61005.

upper part of the coleoptile (figs. 3, 4), decreasing rapidly toward the base. In the upper part of the mesocotyl generally a minimum is found. The coincidence that the minimum for auxin concentration is in the same region as the maximum for growth has already been

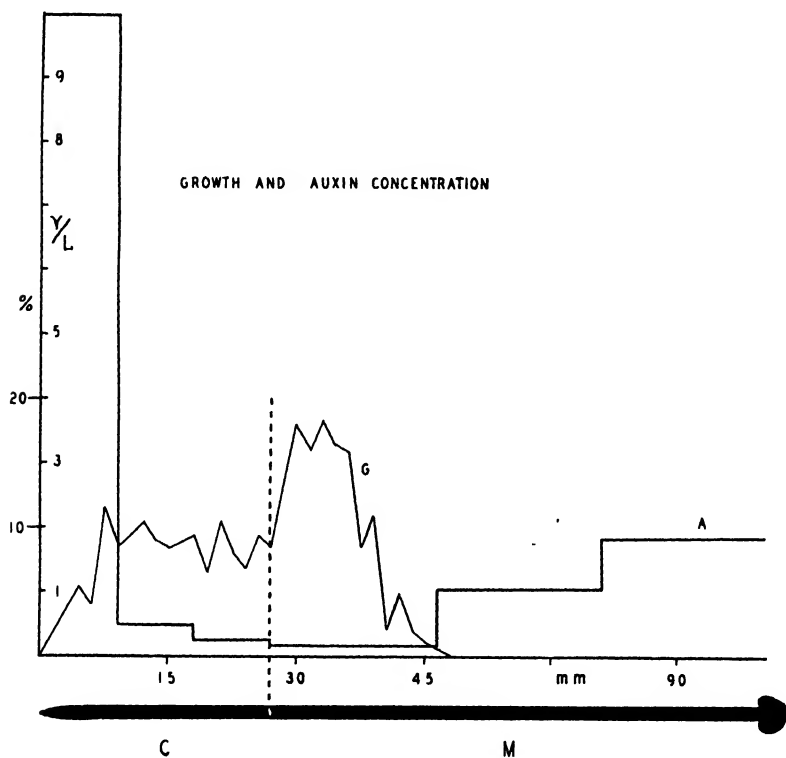


FIG. 3.--Growth and auxin distribution of 5-days-old maize seedlings. Growth in percentage increase in length of 1.5 mm. long segments in 2 hours (curve G). Auxin concentration in gammas indoleacetic acid equivalents per liter water contained in the plant (curve A). Nos. 70201, 71013, 17022, 71020.

discussed, and was attributed to the great sensitivity to auxin in this region.

From the apical to the basal regions of the mesocotyl the auxin concentration increases. In this basal region, at the time the analyses were made, growth had stopped completely and also there is no response to applied auxin (fig. 2). The theory that the decrease in response to auxin in the basal regions of plants is due to their de-

creased auxin content (33, p. 75) does not seem to hold in this case. This relatively high auxin content in the basal regions of the mesocotyl may be linked with the abundant root formation in those regions.

As an explanation of the relatively high auxin concentrations at the basal part of the mesocotyl, the following possibilities may be considered.

1. Auxin produced in the upper parts of the plant may accumulate in the lower portion of the mesocotyl. If this were the case a de-

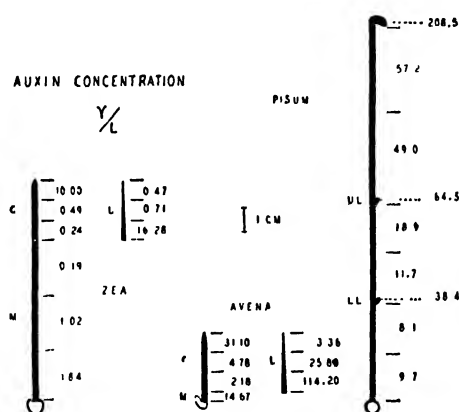


FIG. 4.—Auxin concentrations of etiolated seedlings of corn, oats, and pea, expressed in gammas indolacetic acid equivalents per liter water contained in the plant. Corn was 5 days, oats 3 days, and peas 7 days old when analyzed, which are stages of development at which these plants are mostly used in the laboratory. *c*, coleoptile; *m*, mesocotyl; *l*, primary leaf; *ul*, upper lateral bud; *ll*, lower lateral bud. Buds in reality are inserted at opposite sides of the stem.

creased auxin production should correspond to a decreased amount of auxin in the basal portion of the coleoptile. Table 3 shows the auxin content of intact plants and plants twenty hours after decapitation, and also of plants from which the roots had been removed. Decapitation consisted of the removal of the upper 1 or 2 mm. of the coleoptile tip; in this way the center of auxin production is removed. Later the auxin production is resumed but to a lesser extent. Removal of the root system of a seedling also leads to reduced auxin production (22). With both treatments the auxin content of the coleoptile and the upper region of the mesocotyl is

reduced (table 3). In the base of the mesocotyl, however, the auxin content seems increased. This makes it highly improbable that the relatively high content in the base of the mesocotyl is due to accumulation of auxin produced in the upper regions of the plant.

2. It may be possible that the auxin is carried from the seed into the basal regions of the mesocotyl by the root-pressure stream. The seeds of 5-days-old seedlings carry large amounts of auxin. LAIBACH

TABLE 3

EFFECT OF DECAPITATION AND DEROOTING ON AUXIN DISTRIBUTION OF MAIZE SEEDLINGS. COLEOPTILE TOP OR ROOTS CUT OFF 20 HOURS BEFORE ANALYSIS (NOS. 70903, 6, 8, 9, 13, 16)

PART OF PLANT ANALYZED	AUXIN CONTENT OF 100 PLANTS IN DEGREES OF CURVATURE IN AVENA TEST		
	INTACT	DECAPITATED	DEROOTED
Combined coleoptile and leaf			
Upper half	7.1	4.4	0.4
Lower half	4.6	1.4	0.0
Mesocotyl			
Upper third	5.1	2.1	0.0
Middle third	3.8	3.6	3.0
Lower third	8.9	11.7	11.4
Seed	7.1		
Roots	6.0		

and MEYER (6) found auxin still present in the kernels of 5-weeks-old corn plants. Although auxin may be present in the seed, the root-pressure stream apparently does not affect the auxin content of the basal regions of the mesocotyl (table 3). However, the possibility that active auxin is transported from the seed to the mesocotyl in some other way still exists.

3. An activation of the auxin precursor into active auxin in the middle and basal regions of the mesocotyl may take place. The precursor was studied by SKOOG (11) in *Avena*. It is transported from the seed to the top of the plant. Such a transport might be independent of the root-pressure stream. This would also explain

why the auxin content of the mesocotyl base seems higher in the derooted and decapitated plants than in the intact ones. In the former two groups of plants the total auxin production is reduced; hence less precursor is used, leaving a higher concentration in the basal regions which respond with an increased production of auxin.

Very little attention has been paid to the auxin content of the primary leaves of the seedlings of *Avena* and maize. At the stage of development in which the plants were analyzed this leaf is still inclosed in the coleoptile. It was thought for a long time that the primary leaves of *Avena* did not contain auxin (11). SKOOG was the first to demonstrate auxin in these leaves, by diffusing auxin from the basal part into agar blocks and by testing for auxin with his sensitive deseeded method. The amounts of auxin obtained were so minute that the standard *Avena* test was not sensitive enough to detect them. Thus it was surprising to find that auxin concentrations in the primary leaves of corn and *Avena* seedlings were higher than in any other part of the seedling (fig. 4). The tips of the primary leaves show a relatively low auxin concentration, making the auxin distribution in the primary leaves just the reverse of that in the coleoptiles. The auxin in the leaf must not be readily transportable, which is indicated by SKOOG's experiments and by figures 3 and 4. In these figures it is shown that the minimal auxin concentration is found in the apical region of the mesocotyl. If any appreciable amount of auxin diffused from the primary leaf, the minimum would not be found immediately under its place of attachment.

DISTRIBUTION OF AUXIN IN AVENA SEEDLINGS

THIMANN (14) studied the distribution of auxin in *Avena* seedlings, using his acid and chloroform method. With the ether extraction method and without acidifying, results were obtained which closely correspond to his. Figure 4 shows that in the upper region of the coleoptile a concentration was found corresponding to 31.10 gammas indoleacetic acid per liter water contained in the plant material. From THIMANN's figures (14, p. 32, and 33, p. 68) and from his table V (33, p. 43), it was calculated that the auxin concentration of the upper third of the coleoptile corresponds to values

between 9.4 and 22.2 gammas indoleacetic acid per liter. These values compare favorably with the one (31.10) shown in figure 4. An accurate comparison was impossible because the sensitivity of THIMANN's test plants is unknown. The values shown in figure 4 should not give the impression that the auxin concentrations given there are constants. In the first place, the auxin concentration varies with the stage of development (see for instance figure 5). Then, even though the plants were grown in physiological darkrooms under

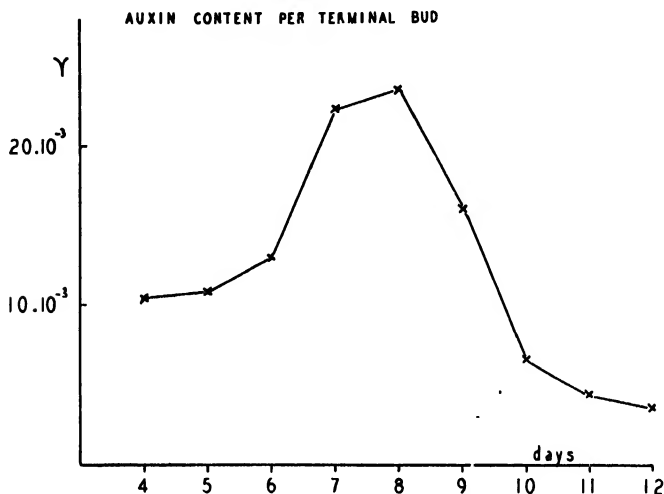


FIG. 5. Auxin content in indoleacetic acid equivalents of terminal pea buds as a function of age of etiolated seedling. At 4 days the buds have just emerged above surface of the sand in which they are planted. Nos. 70920, 70922, 71005, 71006.

constant temperature and humidity, there still was considerable variation in auxin content from day to day. It was found that these uncontrollable fluctuations were much more pronounced in pea plants than in *Avena* seedlings.

For the lower part of the *Avena* seedlings, THIMANN found values which are higher than the ones shown in figure 4. Calculated from his data, values between 3.6 and 8.6 gammas indoleacetic acid equivalents were found. This may be due to his way of analysis. From his illustrations (14, fig. 2 and 33, fig. 30) one can conclude that the mesocotyl was included in the analysis of the basal regions. Figure 4 shows clearly that the mesocotyl has a much higher auxin

content than the basal part of the coleoptile. Mesocotyls of plants grown in glass holders with their roots in water are usually not longer than 3 to 4 mm. The mesocotyl length depends very much upon the amount of light it gets (1, 20). If grown in complete darkness, mesocotyls of several inches long may develop. If grown in sand with the seeds sufficiently covered, mesocotyls develop which are longer than the ones grown in water cultures. Table 1 gives the analyses of two sets of plants, one grown in glass holders and the other in sand. The upper parts (coleoptile and primary leaf) of the plants of both sets are identical as far as their auxin content is concerned. The mesocotyl of the sand-grown plants was 24 mm. long and showed an auxin distribution very similar to that of corn mesocotyls. At the apical regions it is low and in the basal regions of the mesocotyl it is high. No special experiments have been carried out to determine whether the high auxin content of the primary leaf in some way affects the auxin concentration of the mesocotyl. From the distribution of auxin in the mesocotyl of sand-grown plants, however, it appears that this is not the case, and that the high auxin content of the mesocotyl base should be attributed to auxin or its precursor coming from below.

Part II: Auxin concentrations and bud inhibition in *Pisum*

The pea variety Alaska was used throughout these experiments. The seeds were soaked for a few hours in water and planted in moist sand in a physiological darkroom at 24° C. and 90 per cent humidity. At the age of about seven days (from the time of soaking) the plants were ready to be used. The intact pea plants (etiolated) at that time have an auxin content as shown in figure 4. The terminal bud has an auxin concentration which is much higher than the concentrations found in the tips of corn or oat coleoptiles. This was surprising because by the diffusion method much more auxin can generally be obtained from corn and oat tips than from terminal buds of pea seedlings. WENT and THIMANN (33, p. 82) state that only small amounts of auxin have been obtained from apical buds of etiolated pea plants by the diffusion method. As figure 4 shows, the terminal bud has the highest auxin concentration of the etiolated pea seedling. Toward the base the concentration decreases. The dormant

lateral buds have a higher concentration than the adjacent stem tissue. The buds have been extracted without any part of the stem attached to them. The stipules, for technical reasons, were analyzed with the buds.

The auxin content of the seedlings varies with their age. Figure 5 shows the content per terminal bud at stages of development between four and twelve days after the seeds were soaked.

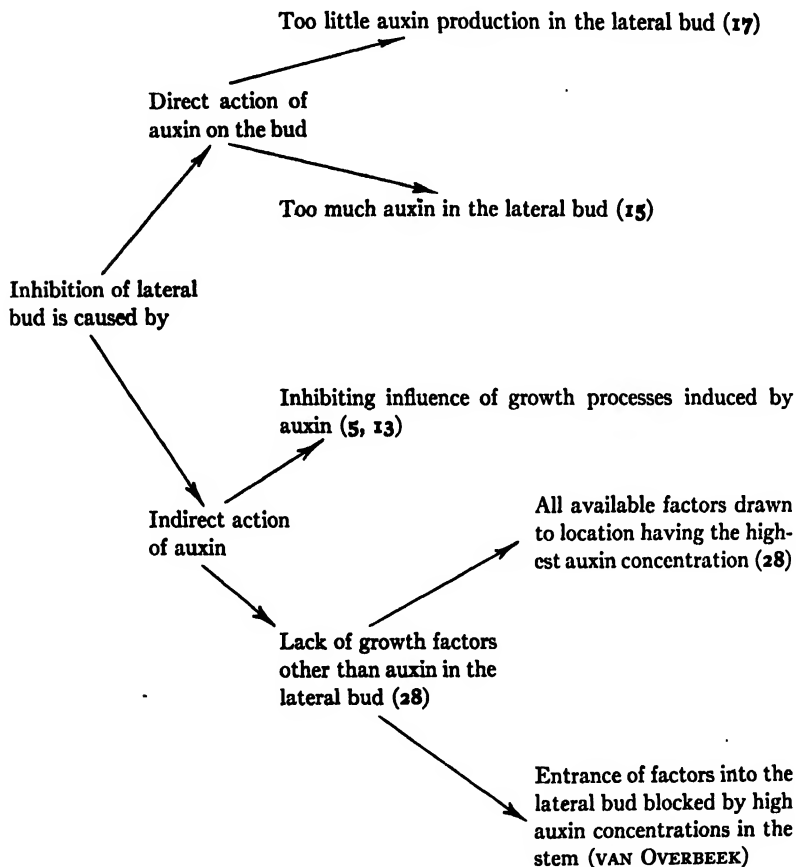
After the terminal bud of a pea seedling is removed, a lateral one starts to develop. In the pea this lateral bud generally is the upper one (figs. 4, 6). This inhibitory influence of the terminal bud upon the lateral ones has been known for a long time; it forms the basis of pruning. THIMANN and SKOOG (16) were the first to demonstrate that this inhibitory influence of the terminal bud is nothing but the auxin produced by it. A little later LAIBACH (5) demonstrated the same. THIMANN and SKOOG removed the terminal bud and put a dosage of auxin on the stump. The buds did not start to grow, but if the auxin was removed the lateral bud developed. This would indicate that auxin is able to prevent lateral buds from developing.

To explain this effect on bud inhibition, several theories have been advanced. Some of them try to explain it on the basis of a direct action of auxin on the lateral bud, others consider the effect of auxin as indirect. The scheme on the opposite page may be used to illustrate these various theories.

There are two groups of theories, one favoring direct action of auxin on the lateral bud and another favoring indirect action. To the first group belong the theories of THIMANN and SKOOG and a recent one of THIMANN. According to the first theory (17), the auxin produced by the terminal bud reaches the lateral buds and prevents their own production of auxin. As soon as the terminal bud is removed the lateral buds commence to synthesize auxin on their own account, which makes the buds develop. The authors of this theory showed that from dormant buds of *Vicia* no auxin could be obtained by the diffusion method. If the lateral buds started to develop large amounts of auxin could be found.

The recent theory of THIMANN (15) postulates that the relation between auxin concentration and growth can be expressed as an

optimum curve. Roots, buds, and stems all have their optimum at different concentrations. According to this theory the buds are inhibited at a concentration of $10^{-6.7}$ molal or higher. The bud growth is stimulated, according to THIMANN's scheme on page 411,



at a concentration lower than $10^{-6.7}$ molal (which corresponds to 37 gammas indoleacetic acid per liter). In other words, when a lateral bud will grow out it must have a lower auxin content than when it stays dormant, according to this theory (15). This auxin content of the lateral bud at different stages of development can easily be determined with the extraction method, and in this way the validity

of the theory (15) can be tested. This is done below; figures 6, 7, and 8 give the results. They show that after decapitation the auxin content and concentration of the lateral buds increases over that in the dormant stage. As soon as twelve hours after decapitation the first increase in auxin content was observed. Figure 6 shows that during

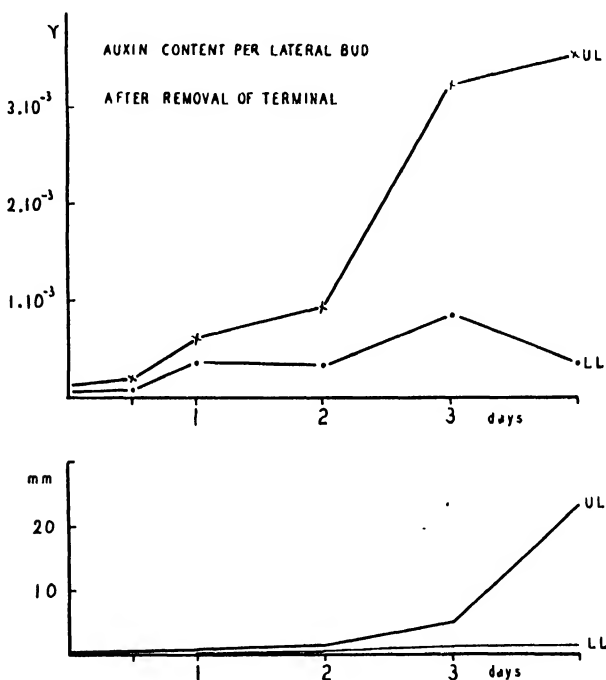


FIG. 6.—Auxin content (indoleacetic acid equivalents) and length of lateral pea buds after removal of terminal bud. Abscissa: time after decapitation of terminal bud. Nos. 70915, 70917, 70920, 70921, 70922, 70923, 70924, 71002, 71007, 71008a.

the first twenty-four hours after decapitation, both the upper and the lower lateral bud increased in auxin concentration. They also increased in weight (table 4). From that time on the upper bud steadily increases its auxin content, while the lower one (the one that does not develop) fails to show further increase. Four days after decapitation the upper lateral bud has an auxin content corresponding to between 3 and 4 $\times 10^{-3}$ gamma indoleacetic acid. This is the amount the terminal bud would have had if the plant had not been

decapitated (11-days-old plants of figure 5). It may be observed that the great increase in growth of the upper lateral bud (fig. 6) is preceded by a sharp increase in auxin content in this bud.

Figure 7 shows the auxin concentrations in pea plants twenty-four hours after decapitation. Three sets with about 150 uniform pea seedlings in each set were selected.

One was kept intact (*N*), another was decapitated about 2 cm. above the upper lateral bud (*D*), a third was also decapitated but followed by application of 0.1 per cent indoleacetic acid in lanolin to the cut stump (*DA*). Of each set, seventy-five plants were analyzed for auxin concentrations while the remaining ones were used for weight and inhibition determinations. The paste containing 0.1 per cent indoleacetic acid proved to give a complete inhibition of the laterals. Figure 7 shows the average result of two identical experiments. The same data are used in figure 8 where the relative changes in auxin due to decapitation and decapitation followed by auxin application are shown (intact plants 100 per cent). The effect

of decapitation is seen by comparing the *N* and *D* curves and the diagrams of figures 7 and 8. In the stem the auxin concentration decreases after decapitation; in the lateral buds it increases.

This is in agreement with the results obtained by THIMANN and SKOOG (17) with *Vicia*. They found that in the undeveloped lateral buds the auxin production was only 1.6 plant units per hour; in the developing laterals it was found to be 19.2. It is this increase in

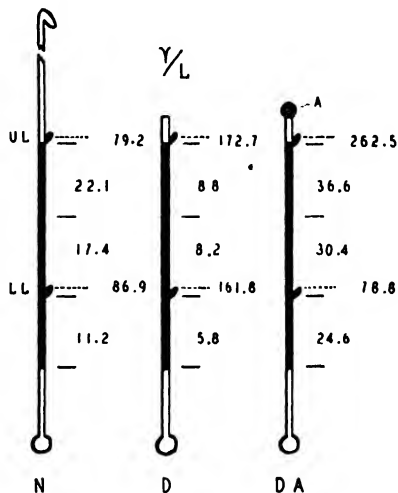


FIG. 7.—Auxin concentration (gammas indoleacetic acid equivalents per liter water contained in the plant) of pea seedlings 24 hours after decapitation and auxin application at cut surface of stump (*DA*), of seedlings that were left intact (*N*), and of seedlings decapitated without additional auxin application. Auxin applied: 1 gamma indoleacetic acid per 1000 gm. of lanolin. Of all the lateral buds shown, upper lateral one (*UL*) of *D* is only one that will develop. Nos. 71007, 71008a, 71014.

auxin production which according to the theory (17) is responsible for the growing out of the lateral buds. From figures 6 and 7 it follows that this increase in auxin production increases the auxin content and the auxin concentration of the lateral buds. Still, any increase in auxin concentration in the lateral buds to a concentration range found in the developing buds does not make the lateral buds grow out. This should be the case if the theory were valid. In fig-

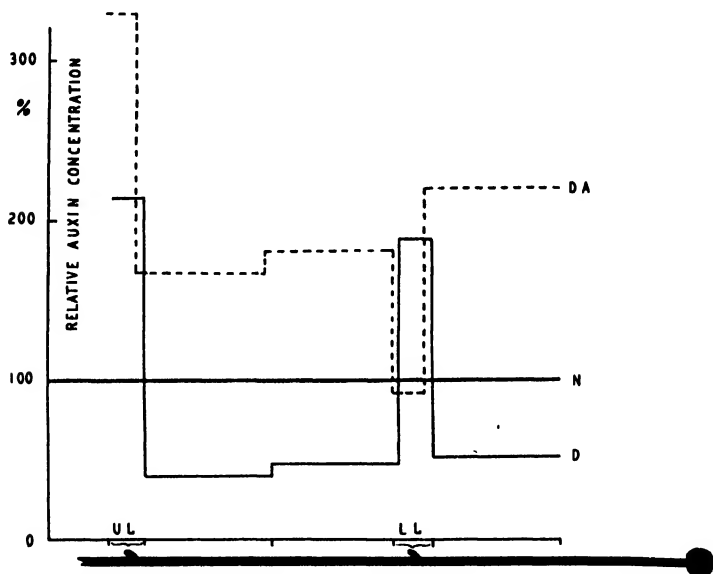


FIG. 8.—Effect of decapitation (*D*) and decapitation followed by auxin application (*DA*) on auxin concentration throughout etiolated pea plants. Auxin distribution shown was found 24 hours after decapitation. Intact plants 100%. Nos. 71007, 71008a.

ures 7 and 8 auxin concentrations are given of plants which were decapitated and on the stump of which auxin dissolved in lanolin was applied (*DA*). The auxin concentration in the stem of these *DA* plants is increased over that in the intact and decapitated plants. The auxin concentration of the upper lateral bud of the *DA* plants is higher than that in the intact plants, and even higher than in the upper lateral bud of the decapitated plants. Nevertheless this upper lateral bud (*DA*) fails to grow out, from which it may be concluded that a mere lack of auxin production, hence lack of auxin, is not the cause of the failure of the dormant lateral buds to develop. This

conclusion is not valid if one assumes that the lateral buds produce a kind of auxin which has a physiological activity completely different from indoleacetic acid. This is extremely unlikely, since one would expect an auxin of the type of auxin-a to be produced in pea plants. The conclusion seems evident that bud inhibition cannot be due to a direct action of auxin on the bud. Hence, bud inhibition can be due neither to relatively high auxin concentrations in the lateral bud

TABLE 4
WEIGHT OF SEVENTY-FIVE LATERAL BUDS 24 HOURS
AFTER REMOVAL OF TERMINAL BUD

	WEIGHT IN MILLIGRAMS	
	INTACT	DECAPITATED
Upper lateral bud.....		
Fresh.....	193.7	283 0
Dry.....	23 1	35 1
Lower lateral bud		
Fresh.....	130 3	181 6
Dry.....	15 4	22 4

nor to relatively low auxin concentrations or low auxin production in these buds.

In figures 7 and 8 it is shown that the effect of decapitation on the distribution of auxin is twofold. The auxin concentration in the lateral buds increases, while in the stem it decreases. Since the bud inhibition of the type discussed here can undoubtedly be controlled by auxin, and since the conclusion was reached that the auxin concentration in the lateral buds themselves does not directly determine whether such bud will develop or not, it follows that a relatively low auxin concentration in the stem may be the important factor responsible for the development of the lateral bud, and a relatively high auxin concentration in the stem apparently prevents the lateral buds from developing. This conclusion is also supported by the following experiments by SKOOG (10). He reduced the auxin content of the plants by irradiating with x-rays, and found that the more auxin destroyed (within certain limits) by irradiation, the bet-

ter was the development of the lateral bud. He excluded the possibility of stimulation of the laterals by the x-rays by shielding the buds.

How a high concentration of auxin in the stem may prevent the development of the lateral buds is the next question to be considered. The following experiments may lead to its explanation. A ring of lanolin paste in which 0.1 per cent indoleacetic acid had been dissolved was applied around the stem below the lateral bud of decapitated pea plants. The lateral bud developed. If a ring of the paste was applied 2 cm. above the upper lateral bud this bud also grew out. If, however, the paste was applied at the apical cut surface 2 cm. above the upper lateral bud, this bud was completely inhibited. The conclusion may be drawn that in order to be effective the auxin (of the concentration employed) must be introduced in the vascular system of the stele. The epidermis, cortical tissue, and endodermis apparently impede the auxin transport in a lateral direction to a considerable degree. It is clear that auxin of a concentration higher than the one employed here may very well be able to inhibit lateral buds when applied in a ring around the stem above these buds.

Another experiment to be considered is the one already shown in figure 7. If the auxin concentration of the lower lateral bud of the *DA* set is compared with that of the same bud of the *N* set, it is seen that these concentrations are practically the same. Repetition of the experiment brought the same results. Apparently a relatively high auxin concentration in the stem prevents the auxin formation in the lateral bud. To study this, one must be able to distinguish between the auxin produced by the bud and the auxin introduced by application. This was found possible by using phenylbutyric acid instead of indoleacetic acid. WENT found that this substance has not the slightest activity in the *Avena* test, yet it is able to inhibit bud development to a considerable degree (table 5). WENT (30) found that there are several substances which have only part of the properties found in auxins. With this phenylbutyric acid an experiment similar to the one of figure 7 was made. Its result is recorded in table 6. The phenylbutyric acid had to be applied in rather high concentrations in order to be effective. Furthermore, it does not penetrate

into the plant as readily as does indoleacetic acid. Applied at a distance of 2 cm. above the upper lateral bud it is hardly effective. Applied at about 1 cm. above the upper lateral bud it inhibits de-

TABLE 5
LENGTH IN MM. OF LATERAL BUDS OF PLANTS OF TABLE
6 MEASURED FOUR DAYS AFTER DECAPITATION
AVERAGE OF 30 PLANTS EACH

LATERAL BUD	N	D	DP
Upper	0	18 5	6 7
Lower	0	0 7	6 9
PERCENTAGE HAVING LENGTH BELOW 2 MM.			
Upper	100	0	38
Lower	100	86	20

TABLE 6
AUXIN CONCENTRATIONS OF THREE SETS OF ETIOLATED PEA
SEEDLINGS. *N*, INTACT; *D*, DECAPITATED; *DP*, DECAPI-
TATED FOLLOWED BY APPLICATION OF 2% PHENYLBUTYRIC
ACID PASTE. ANALYSES MADE 24 HOURS AFTER DECAPITA-
TION. CUT SURFACE AND PASTE APPLICATION 1 CM.
ABOVE UPPER LATERAL BUD. CONCENTRATIONS IN
GAMMAS INDOLEACETIC ACID EQUIVALENTS PER LITER
(NO. 71208)

	N	D	DP
Upper lateral bud	104.3	277 0	94 0
Upper part of internode between buds	9.4	4.2	0 98
Lower part of same internode	1.37	1 8	0.70
Lower lateral bud	0 0	0.0	156.3

velopment of this bud to a marked degree. Table 6 shows that the auxin concentration in the upper lateral bud of the decapitated plants to which phenylbutyric acid had been applied does not increase. Hence the formation of auxin in the lateral bud is prevented

by a high concentration of this acid. This confirms the preliminary conclusions drawn from the low auxin concentration of the lower lateral bud of figure 7 *DA*,¹ that a relatively high concentration of auxin in the stem prevents the formation of auxin in the lateral buds.¹

The lower lateral buds of the plants treated with phenylbutyric acid (table 6 *DP*) show a high auxin content compared with the lower buds of the sets *N* and *D*, which was abnormally low in this series of plants. In the table the auxin content of these buds is given as 0.0; this does not necessarily mean that the buds were devoid of auxin, but that for the number of buds extracted (seventy-five) and for the volume of agar in which the dry extract was taken up (0.5 cc.) no curvature could be obtained in the *Avena* test. It may well be possible that if the number of buds extracted had been larger a small curvature would have been obtained. It was mentioned in the first part of this paper that even though the seedlings were grown under controlled temperature and humidity conditions, still considerable fluctuations in growth and auxin content could be observed from day to day.

The relatively high auxin content of the lower lateral bud of the *DP* set may be accounted for as follows. If less precursor is used for the auxin production of the upper lateral bud (*DP*) than for the more rapidly developing upper lateral bud of the set *D* (in the set *N* the precursor is used for auxin production in the terminal bud), more is left over for the auxin production of the lower lateral bud of the plants of the set *DP*. The lower lateral bud is able to develop because, as has been shown, the phenylbutyric acid penetrates with difficulty into the stem and is not present in high enough concentrations in the lower part of the plants to prevent bud development.

If one assumes that the auxin production in the lateral buds does not take place because auxin precursor is lacking, the problem is reduced to the following considerations: Relatively high auxin concentrations in the stem prevent the auxin precursor from entering the lateral buds. If the auxin precursor cannot enter the bud it is also likely that other factors necessary for growth of the buds are not

¹ FERMAN (Rec. Trav. Bot. Néerl. 35: 177-287. 1938) came to the same conclusion in a different way. Similar conclusions may be drawn from SKOOG's (Amer. Jour. Bot. 25: 361-372. 1938) table 5.

able to enter. WENT has assumed that bud inhibition is due to lack of specific growth factors (see scheme on page 149). He believes these factors to be hormones. For the present consideration the nature of those growth factors is of secondary importance. With grafting experiments, WENT (29) showed that the factors move up through living tissue of the vascular system. The vascular system of the pea at the stages of development as used in the present experiments has been studied by GOURLEY (3). He found that in the stele a number of large vascular elements are present. The dormant lateral bud is connected by means of only a small trace to this stelar system. Cortical bundles have no connection with the dormant lateral bud. MORELAND (8) showed that in bean plants the vascular supply leading to the cotyledonary buds is normally very weakly developed. He also showed that within forty-eight hours after decapitation this vascular supply has been increased.

It has already been shown that a high concentration of auxin in the stem prevents the lateral buds from developing, and that in order to be effective the auxin has to be introduced to the vascular system. So one might conclude that a relatively high auxin concentration in the vascular system of the stem is necessary for inhibition of the lateral bud.

One way to visualize the action of auxin in the vascular system is the following. Auxins are surface active substances. Hence, by entering the vascular system their molecules may get adsorbed to the walls of the vascular elements (phloem or xylem or both).¹ Suppose the materials necessary for growth are translocated from the seed to other parts of the plant through the vascular system by means of a mass flow. The velocity of such a flow will be affected by the properties of the wall of the vascular element and by the diameter, as the flow of water through a capillary tube is affected by the properties of the wall and the diameter. If the auxin is adsorbed to the wall of vascular elements, no doubt the physico-chemical properties of the wall will be changed; hence the mass flow through such a vascular element will be changed.

That auxin has an effect on the flow of protoplasm in cells is beyond doubt. This has been shown by THIMANN and SWEENEY

¹ The phloem is likely to be the most important in this consideration.

(18), who showed that in low concentrations auxins promote the velocity of protoplasmic streaming; in high concentrations the streaming is slowed down. If one assumes that the flow of food materials through the phloem elements is slowed down likewise by high auxin concentrations, then it will be clear that the effect of auxin (adsorbed on the wall) will be greater in small narrow elements than in wide ones. In other words, the flow of food material will be only slightly interfered with in the main bundles of the stele but through the narrow trace to the dormant lateral bud it will be impossible. Thus growth factors may be prevented from reaching the dormant bud, but allowed to pass through the large bundles to the growing region of the stem.

The validity of this conclusion can be tested in the following way. If the laterals are allowed to develop, the vascular system develops with them. MORELAND (8) showed that decapitation of the terminal bud or any other treatment leading to development of the lateral buds is followed by increased vascular supply to the lateral buds. He showed that prior to expansion of the buds their vascular connections have been strengthened. The effect of auxin on lateral buds with such an increased vascular supply, on the basis of the preceding considerations, can be expected to be less the more the lateral bud has developed. This indeed is what happens. It was known to THIMANN and SKOOG that after the laterals had grown out to a certain length, auxin when applied to the main stem was without effect on these laterals. Figure 9 shows how the growth of the upper lateral buds is influenced by auxin applications (in the way of figure 7 *DA*), made when the laterals had reached different stages of development. Curve *D* is the growth curve of laterals of decapitated plants to which no auxin had been applied. Curves 0, 1, 2, and 3 are growth curves of decapitated plants to which 1 per cent indoleacetic acid in lanolin was applied after the laterals had been allowed to grow for respectively 0, 1, 2, and 3 days. It is clear that the effect of applications of auxin to the stump of the stem is less the more the growth of the lateral has advanced. This not only is elongation of the lateral bud, because two days after decapitation, when the auxin is hardly able to influence the growth, the lateral bud has barely increased in length (fig. 6). What is important here is that prior to

expansion of the lateral bud its vascular supply has been increased, as found by MORELAND.

The experiment of figure 9 was also done with auxin pastes containing 0.1 and 0.02 per cent indoleacetic acid. The results were the same, although of course the inhibition is much less with weaker

DECREASED AUXIN EFFECT ON DEVELOPING

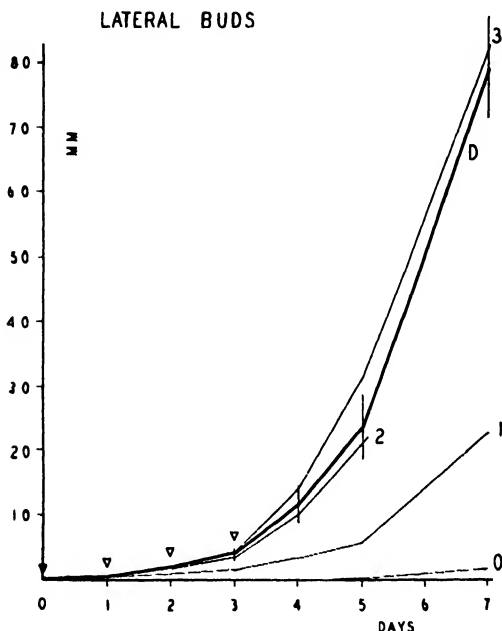


FIG. 9.—Length of upper lateral bud in relation to time after decapitation. Indoleacetic acid applied to cut surface of stump after buds had been allowed to develop for 0, 1, 2, 3, days (curves 0, 1, 2, 3). Heavy curve *D* is of plants to which no auxin was applied. Indoleacetic acid paste 1%. Inverted triangles indicate time of auxin application in the various sets. Thin vertical lines indicate range of variation for curve *D*. Averages of about 30 plants. No. 71220.

pastes than with stronger ones. The plants of curve 2 were taken out of the experiment after the fifth day (abscissa) and analyzed for their auxin concentrations. The results are shown in figure 10. In the decapitated plants without auxin paste the highest auxin concentration is found in the top of the developing lateral. In the other cases, especially in the one where 1 per cent indoleacetic acid was

applied, the highest auxin concentration is found in the upper part of the main stem. If a situation like this exists, then according to WENT's theory (see scheme on page 149) all the growth factors limiting the growth of a lateral bud move to the site of the highest auxin concentration, which is the upper part of the main stem. Hence, according to this theory the laterals of the set *DA 1* of figure 10 should be inhibited. This does not happen.

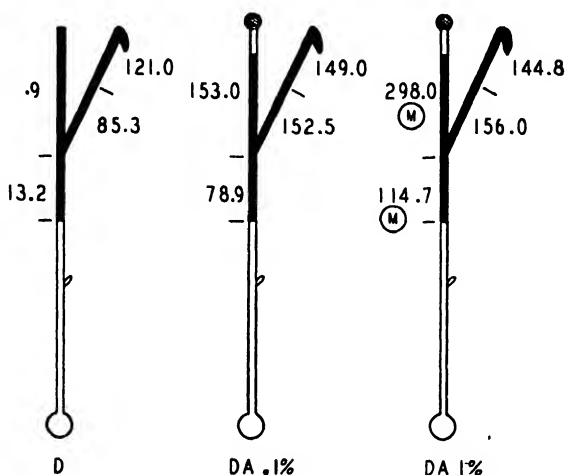


FIG. 10.— Auxin distribution (gammas indoleacetic acid equivalents per liter water contained in the plant) of pea plants to which auxin had been applied after laterals had been allowed to develop for 2 days. Analyzed 3 days after auxin application. In set *D* no auxin was applied. In *DA 0.1*, 0.1% indoleacetic acid was applied and in set *DA 1*, 1% was applied. The *DA 1* set was analyzed where curve 2 of figure 9 is terminated. The *M* in circle next to a figure means that given value is too low because of a maximum angle in the assay. No. 71221.

SNOW (13) assumes that the formation of an inhibitory substance caused by the action of auxin is a growth process. He investigated the lateral buds of *Vicia* and pea plants grown in light, and believes that the inhibiting substance is able to spread throughout the plant regardless of polarity. He based this view on the assumptions that auxin is unable to move in the plant in a morphologically upward direction, and that the base of the inhibited shoot of his plants is free of auxin. Figure 10 shows that the auxin applied at the top of the main stem may be able to move upward in the developing lateral.

Indeed LE FANU (7) has not been able to obtain auxin by diffusion from inhibited pea shoots. This, however, does not prove that the shoot does not contain auxin. The experiments with primary leaves of *Avena*, mentioned in the first part of this paper, have shown that large amounts of auxin are present in these leaves although only extremely small amounts can be obtained from them by diffusion.

In the pea seedling as a rule the upper lateral bud develops after decapitation while the lower remains dormant (fig. 6). This may be explained by the following considerations. Table 7 shows the results of an experiment in which the stem of the pea seedling was either cut

TABLE 7

PERCENTAGE OF LATERAL BUDS THAT HAVE STARTED TO DEVELOP WITHIN TWO DAYS AFTER MAIN STEM HAD BEEN CUT OFF 2 CM. ABOVE EITHER UPPER OR LOWER LATERAL. AVERAGES OF ABOUT 40 PLANTS

DECAPITATION	NUMBER	PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID PASTE				
		0	0.005	0.01	0.05	0.1
Above upper lateral bud (upper buds).	{ 71222	74	81	67	5	0
	{ 71228	81	71	22	0
Above lower lateral bud.....	{ 71222	21	13	5	0	0
	{ 71228	20	14	0	0

off 2 cm. above the upper lateral bud or 2 cm. above the lower one. If no paste containing auxin was applied to the stump, of the upper buds about 75 per cent had started to grow out within the first two days after decapitation, while only 20 per cent of the lower buds were developed in the plants which were decapitated 2 cm. above this bud. So the upper bud is about two days ahead in development. From figure 6 it may be seen that this is exactly the stage at which the upper bud starts to produce large amounts of auxin, which flows into the main stem (fig. 10D) where it raises the concentration to a point where it inhibits growth of the lower lateral bud. There is also evidence that low auxin concentrations are more effective in inhibiting bud development of the lower lateral bud than of the upper. This evidence is presented in table 8. Here two experiments are given. In

experiment 71222 an auxin application of 0.01 per cent indoleacetic acid in lanolin does not inhibit development of the upper lateral bud, while the length of the lower one is reduced to 50 per cent of the length of the controls. In experiment 71228 a similar effect is seen at a still lower concentration.

In conclusion, it may be helpful to summarize the facts available for the explanation of bud inhibition. After decapitation, two changes take place in the auxin content of a seedling: (1) the auxin production (17) and concentration (figs. 7, 8) of the lateral bud in-

TABLE 8

RELATIVE LENGTH OF UPPER LATERAL BUD AFTER REMOVAL OF STEM UP TO 2 CM. ABOVE THIS BUD (*U*), AND OF LOWER LATERAL BUD AFTER DECAPITATION 2 CM. ABOVE (*L*). INDOLEACETIC ACID APPLIED IMMEDIATELY AFTER DECAPITATION ON CUT SURFACE OF STUMP

EXPERIMENTAL NO.	TIME AFTER DECAPITATION IN DAYS; AND <i>U</i> OR <i>L</i> SET	PERCENTAGE CONCENTRATION OF INDOLEACETIC ACID PASTE				
		0	0.005	0.01	0.05	0.1
71222	3 (<i>U</i>)	100	108	95	28	0
	3 (<i>L</i>)	100	104	54	20	0
	4 (<i>U</i>)	100	124	112	28	0
	4 (<i>L</i>)	100	100	54	20	0
71228	3 (<i>U</i>)	100	107	50	27
	3 (<i>L</i>)	100	65	60	30	...
	4 (<i>U</i>)	100	107	55	24
	4 (<i>L</i>)	100	80	65	30

creases; (2) the auxin concentration in the stem decreases (figs. 7, 8). The increase in auxin content of the lateral buds, however, is not the direct reason for the development of these buds (fig. 7*DA*), thus ruling out a direct action of auxin on the lateral bud as the cause of bud inhibition, and making it likely that the controlling action is on the stem rather than on the lateral bud itself. A reduction of the auxin content of the stem by other means than decapitation also was shown (10) to result in development of the lateral buds. It was shown that auxin must be introduced into the vascular system of the stele in order to be most effective. This indicates that the place of action from which the auxin controls development of the lateral

buds must be this vascular system. From the anatomy of the pea seedling it is known (3) that the dormant lateral bud is connected with the main bundles of the stele only by means of a small trace. It was shown (table 6) that high auxin concentrations in the vascular system prevent auxin formation in the lateral buds. It was assumed that this auxin formation did not take place because its precursor was lacking, and that the precursor could not pass through the narrow trace. Since this lack of precursor or auxin was shown not to be the cause of the bud inhibition, it was assumed that the flow of materials necessary for growth (including precursor) is stopped by high auxin concentrations. Such a flow could take place in the phloem. It is known (18) that the flow of protoplasm (protoplasmic streaming) is inhibited by high concentrations of auxin; a similar effect may take place in the vascular bundles on the mass flow. If one assumes that the auxin is adsorbed to the walls of the vascular elements, and from there by means of changing the physico-chemical properties of this wall exerts an effect on the flow of growth materials, it is clear why auxin would be able completely to inhibit translocation through narrow vascular elements and hardly at all in larger wider ones. Thus lateral buds could be inhibited while at the same time growth in the main stem could take place. Support of this view was obtained from the following facts. It was known (8) that any treatment leading to development of the lateral buds is followed by an increase in the vascular supply to these buds. This takes place before the bud grows out and within forty-eight hours after the treatment. Auxin was applied to the stump of plants with lateral buds having such an increased vascular supply and was found to be unable to inhibit the development of these buds (fig. 9).³

Summary

1. A method is described by which auxin was extracted from plant materials without the usual addition of acid. Acid was found to

³ After this paper had gone to press, two articles by FERMAN (FERMAN, J. H. G., Proc. Kon. Akad. Wetensch. Amsterdam 41:167-180. 1938; Rec. Trav. Bot. Néerl. 35:177-287. 1938) on the bud inhibition in *Lupinus* were received. This author offers two different explanations, one for the inhibition of intact plants and another for the inhibition with artificial auxin. In intact plants the auxin precursor "is transported acropetally and chiefly attracted to those spots where auxin is most intensely pro-

reduce the yield in some cases (corn) and in other cases (oats) to be of no benefit. The distribution of auxin concentrations in etiolated seedlings of corn, oats, and peas was determined (fig. 4). The amounts of auxin were not expressed in the usual way in arbitrary units, but in indoleacetic acid equivalents which are independent of the sensitivity of the test plant, of the test plant itself, and of the conditions under which the test is made.

2. In corn the distribution of growth was compared with the distribution of auxin (fig. 3). It was found that the region having the greatest growth rate (the upper part of the mesocotyl) has the lowest auxin concentration. This was found to be due to the great sensitivity to auxin in this region.

3. The highest auxin concentrations in the seedlings of corn and oats are found in the basal regions of the primary leaves. This may not be free moving auxin. The average auxin concentration is lowest in corn seedlings and highest in pea plants.

4. The problem of bud inhibition was analyzed in pea seedlings. The effect of decapitation on auxin concentrations in the plant was determined. It was shown that as soon as twelve hours after decapitation the auxin concentration in the lateral buds is increased (figs. 6-8). In the stem the concentration decreases after decapitation. Auxin application increases the concentration in the stem and also in the upper lateral bud. From these analyses it was concluded that a direct action of auxin on the lateral bud is excluded as a possible explanation for its dormancy.

5. From experiments with phenylbutyric acid it was concluded that high concentrations of auxin (or phenylbutyric acid) in the stem prevent the formation of auxin in the lateral bud (table 6).

6. Auxin is most effective in inhibiting lateral buds when introduced into the vascular system of the stele.

duced." The young axillary buds "remain deprived from the precursor and therefore dormant, since they cannot produce auxin and consequently cannot grow out." For the bud inhibition caused by artificial auxin, FERMAN assumes that indoleacetic acid "prevents or seriously hampers already in the basal parts of the stem the upward movement of the precursor in its tracks of transport." Since both explanations ultimately are based on the thesis that it is the lack of auxin which prevents the lateral buds from developing, they can be criticized in the same way as THIMANN and SKOOG's (17) theory.

7. Auxin applied to the stump of decapitated plants some time after decapitation is less effective in bringing about bud inhibition the longer the time between decapitation and application (fig. 9).

8. The preceding evidence, in combination with many valuable data obtained from the literature, has provided material with which an attempt was made to explain the auxin controlled bud inhibition in agreement with all known data. A brief explanation as to how the mechanism of this bud inhibition might work, together with the main facts upon which it was based, is given at the end of part II.

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ANATOMY OF AUXIN TREATED ETIOLATED SEEDLINGS OF PISUM SATIVUM

FLORA MURRAY SCOTT

(WITH TWENTY-NINE FIGURES)

Introduction

The anatomy of auxin induced swellings in *Phaseolus* has been described in detail by KRAUS (4) and his collaborators, and BORTHWICK (1) and his colleagues have given a full account of similar tumors in *Solanum*. The present investigation is concerned with the reactions of the stem of the etiolated seedling of *Pisum sativum* on treatment with a known auxin, indole(3)acetic acid.

Material and methods

Three series of experimental plants were grown: series A and series B were treated with auxin paste, while series C was treated with aqueous solutions of auxin. The seedlings were grown and treated under standard experimental conditions in the laboratory.

SERIES A.—Seeds of the garden pea, *Pisum sativum* (variety Alaska), germinated and grown in the dark under constant conditions of temperature and humidity (24° C. and 85 per cent humidity), developed into typical etiolated plants of the type used by WENT (7) in the standard pea test. When the first foliage leaves began to unfold at the fourth internode, the seedlings were decapitated 1 cm. below this node and the cut surfaces smeared thickly with lanolin-auxin paste (1 gm. indole(3)acetic acid in 100 gm. lanolin). Blunt conical swellings developed in the course of a few days, in the tissues of which root primordia arose (fig. 1).

Control seedlings were grown under exactly similar conditions of darkness and temperature. Some were decapitated and left untreated, others decapitated and treated with lanolin, while a certain number were allowed to continue normal development. No control seedling stem showed any trace of swelling beneath the cut surface. In the treated seedlings, on the other hand, increase in diameter was apparent within the first twelve hours. In seventy-two hours a club-

shaped swelling extended about 8 mm. down the stem, and continued to enlarge during the next few days. The vascular tissues of the control seedlings, decapitated and non-decapitated, developed in the normal way, but differed markedly in extent. A comparison of typical transverse sections of decapitated control and auxin treated internodes indicates this contrast clearly (figs. 23, 24).¹ Collections of seedlings, both treated and control, were made at eight hour intervals up to ninety-two hours, and thereafter less frequently up to 164 hours. The material was infiltrated with Flemming's or with Navashin's solution, imbedded, sectioned, and stained with safranin and fast green or with haematoxylin. Hand sections, treated with iodine potassium iodide, with thionin, or with phloroglucin and hydrochloric acid, were used in the examination of the starch content and the lignification of the endodermis and other tissues. The sugar content was also tested by means of Fehling's solution. Since sugar was abundant throughout the duration of the experiment, this test was of no value from a quantitative standpoint.

SERIES B.—A second series of seedlings was grown to test the effect of age on the swelling of the internode. The seedlings were grown under constant conditions as before, but were not decapitated and treated until they were a week older. Swelling and root formation were slight or even entirely lacking in many specimens (fig. 2).

SERIES C.—Auxin in aqueous solution was used in this series, for a comparison of auxin-water and auxin-paste induced swellings. Various concentrations of auxin were used: (1) 100 mg. per liter; (2) 200 mg. per liter; (3) 400 mg. per liter; (4) 1000 mg. per liter. The resultant swelling and root formation varied markedly according to concentration (figs. 3, 4).

Observations

ANATOMY OF THIRD INTERNODE

The anatomy of the seedling pea, Champion of England, has been described in detail by GOURLEY (2). The root-stem transition region extends through the first two internodes, and typical stem structure becomes established only in the third. The stele in the transition

¹ I am indebted to Mr. R. PLATT of this Institution for making the necessary photomicrographs.

region is anomalous in structure, comprising both exarch and endarch bundles.

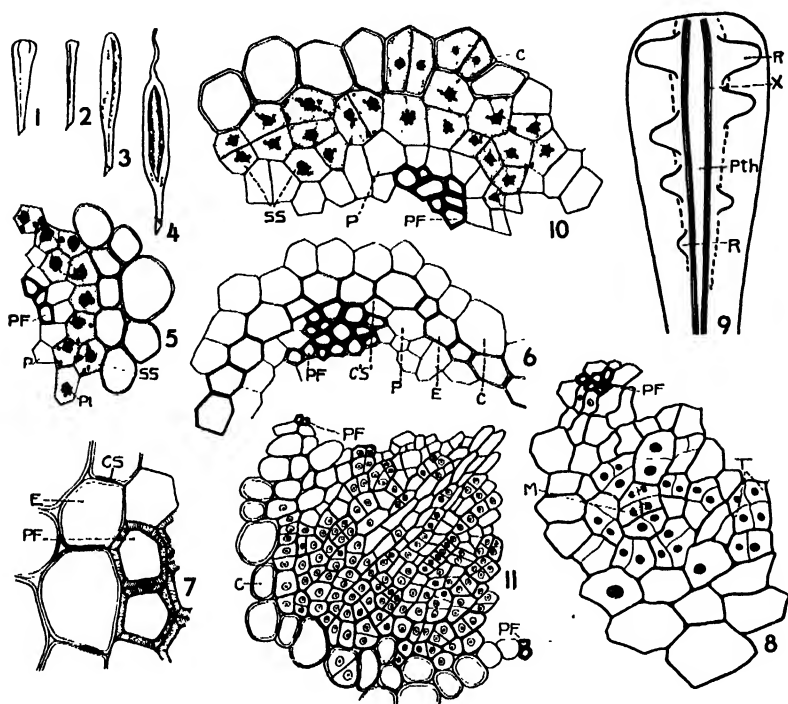
The variety Alaska resembles in essentials the variety Champion of England, so that only a brief outline of structure is necessary here. In the upper part of the third internode, the region of decapitation and treatment with which we are immediately concerned, stem structure is as a rule definitely established.

At this level the stem is still elliptical in outline. Pith and cortex are well developed. The fibrovascular system is made up of a central stele, two cortical fibrovascular bundles, and two fiber strands. Stele, cortical bundle, and fiber strand are each delimited by a starch sheath. The bundles of the central stele, six in number, two polar and two paired laterals, differ in extent and development, obvious in the number of xylem vessels, and they are separated by parenchyma rays. Both polar and paired lateral bundles are capped by groups of pericyclic fibers. The central bundles supply the leaf traces for the fourth and succeeding nodes. The cortical fibers follow the traces out into the rudimentary leaf blades, while the cortical vascular bundles, which are amphiphloic at this level, diverge into the stipules.

At the beginning of experimentation the seedling internode consists of primary tissue. Within twenty-four to thirty-six hours, however, some cambial activity is evident in the seriation of the intrafascicular differentiating xylem and phloem elements. The component cells of the tissues include epidermal, collenchyma, and parenchyma elements; starch sheath cells; pericyclic fibers only slightly thickened and pericyclic parenchyma; sieve tubes, companion cells and phloem parenchyma; spiral and annular, and later pitted elements constituting the xylem.

In unimbedded material the presence of starch in the innermost layer of the cortex serves to define the limits of the stele, the endodermis. In order to facilitate description, the term starch sheath is used for this limiting layer when the starch-containing plastids are in evidence, while the term endodermis indicates that differentiation of the Casparian strip has occurred. At the beginning of experimentation the starch sheath cells are vacuolated, and the starch-containing plastids are grouped as usual around the central nucleus (fig. 5).

Here and there plasmolysis typical of an endodermal cell is noted; that is, the protoplast, attached firmly to the radial walls, shrinks



FIGS. 1-11.* Figs 1-4, variation of form of swellings in auxin-paste and auxin-water series: 1, series A, typical swelling (164 hours); 2, series B (160 hours); 3, series C, 200 mg. per liter (160 hours); 4, series C, same with 1000 mg. Fig. 5, A.P. (24 hours) transection, swelling near tip; starch sheath begins to divide. Fig. 6, A.P. (164 hours) transection below swelling; endodermis complete and Casparian strip typical except at C' , S' . Fig. 7, A.P. (164 hours) transection, base of swelling; variation in extent of lignification of Casparian strip. Fig. 8, A.P. (48 hours) transection near cut surface; cell division in ray cells, mitotic figures, and "tetrad" appearance. Fig. 9, A.P. (140 hours) diagrammatic longitudinal section showing origin of root primordia at successive levels. Fig. 10, A.P. (81 hours) transection near base of swelling; division of starch sheath cells. Fig. 11, A.P. (92 hours) transection, young primordium.

* Abbreviations: A.P., series A. Seedling internodes treated with auxin paste for varying lengths of time, stated in hours. A.P. 2, series B. One week older than A, treated with paste in same way and grown under same conditions. W.S., series C. Same age as A, treated with aqueous solutions of auxin of varying concentrations. C, cortex; C_S , Casparian strip; C' , S' , atypical lignification of strip; E, endodermis; L, limit of meristem cylinder; M, mitotic figure; P, pericycle; PF, pericyclic fiber; Pl, plastid; Ph, phloem; Pth, pith; SS, starch sheath; T, "tetrad" formation; R, root primordium A, B, etc.; X, xylem.

away from the outer and inner tangential walls. This condition is rare, however, and apparently bears no relation to cell position in

regard to rays or vascular strands. Later, in older seedlings (140-164 hours, control and treated) the starch sheath assumes the character of the endodermis of a root, and a Casparian strip is differentiated along the radial walls (fig. 6). The Casparian strip, however, is not visibly thickened as in some root endodermal cells. It is marked merely by a localized lignification of the middle lamella and of part of the primary wall (fig. 7). The secondary wall remains free of lignin material. Lignification is indicated with thionin, and tested with phloroglucin and HCl. Neither Sudan III nor di-methyl-amino-azo-benzene, however, indicates anything more than a faint possible trace of suberin in the modified region of the wall. The development of a functional endodermis in etiolated seedlings has been discussed at some length by PRIESTLEY (5). In *Pisum* the differentiation begins in the lowest internode and proceeds gradually upward toward the stem apex.

The differentiating phloem includes sieve tubes, companion cells, and phloem parenchyma. The sieve tubes are narrow, about one-third the diameter of the adjacent parenchyma cells, and they occur either singly or in groups of two or three. The pitted terminal sieve plates may be determined under oil immersion, and since the walls of the sieve tube stain deeply with fast green, they are distinguishable from the adjacent lighter companion cells.

ANATOMY OF SWELLINGS (SERIES A)

Tissues react to treatment with growth substances (1) by cell expansion (seen principally in the cortex and the pith); and (2) by cell division, notable in the inner cortex, endodermis, pericycle, ray parenchyma, cambial tissue, phloem parenchyma (primary and secondary), and xylem (to a slight extent).

Meristematic activity culminates in the production of root primordia. The seedlings collected at eight hour intervals give a connected picture of the development of the swellings and the included roots.

While development is a continuous process, it may for convenience be considered in four phases. These follow in succession, overlapping of course, but each characterized by a certain dominant activity.

This sequence, although general, is by no means inalterable, and a certain amount of individual variation occurs.

1. Swelling of parenchyma cells, particularly in the cortex and the pith, and infiltration of lanolin into intercellular spaces and spiral vessels (0-48 hours).

2. Formation of a meristem cylinder (16-72 hours).

3. Initiation and development of root primordia (56-164 hours).

4. Lignification of tracheids (116-164 hours).

1. SWELLING OF PARENCHYMA CELLS.— During the first twenty-four hours a definite expansion of the axis is observed which is due mainly to enlargement of the cortical cells. The latter, next to the cut surface, round off and may project as a raised rim around the non-extended vascular bundles and fibers. The parenchyma of the pith, the rays, and the parenchymatous elements of xylem and phloem react in essentially the same way but to a lesser degree. The net result is the rounding of the cut surface as a whole. The zone of maximum expansion in the present instance lies 1-2 mm. below the cut, but the level of this zone depends on auxin concentration. This fact is clearly seen in the aqueous auxin series C (figs. 1-4).

Enlargement of intercellular spaces accompanies cell expansion and allows of the physical penetration of the auxin paste into the tissues, which penetration may be seen by staining with Sudan III. In the first eight hours the intercellular spaces of the cortex are already blocked 3-4 mm. below the cut surface, and the same is true for the pith. Penetration keeps pace with the swelling, and in forty hours the narrow intercellular spaces of the outer cortex and the wider spaces of the inner cortex of the entire segment (1 cm. in length) are practically completely infiltrated. At the same time lanolin is drawn down into the cut spiral vessels to a depth of 7-10 mm. in the first eight hours. At the end of twenty-four hours most of the vessels in the 1 cm. segment are completely blocked (fig. 25).

Older seedlings were examined in order to determine the maximum depth of lanolin penetration. In the cortex this usually does not extend beyond the first centimeter, but in a few of the vessels the lanolin may reach a depth of 17 mm. Of the vessels so blocked, some are narrow, some wide in lumen, so that infiltration is not a function of vessel diameter. Metaxylem elements in general are unblocked,

but a few elements next to the cut surface, in seedlings treated for forty hours, contain intermittent lanolin plugs.

Penetration of intercellular spaces occurs in both control lanolin and in lanolin-auxin seedlings. As already stated, in the former no swelling of the cut internodes results.

2. FORMATION OF MERISTEM CYLINDER.—Active cell division is indicated: (a) by the presence of small, thin walled cells with relatively large nuclei, as in typical meristematic dividing tissue; and (b) by the position and relative thinness of newly formed cell walls in the larger vacuolating dividing cells. This is particularly obvious in the parenchyma cells of the cortex and the rays. In these tissues the outline of the parent cells is distinct, while the daughter cells within are grouped sometimes as tetrads, but more frequently in less regular formation (fig. 8).

Within twenty-four hours meristem activity is beginning near the surface. Twenty-four hours later the following tissues are involved in active division: inner cortex, starch sheath, pericycle, phloem parenchyma, ray tissue, the cambial region, and to a limited extent the marginal xylem parenchyma. The result is a hollow cylinder of meristem encircling the xylem and the inactive pith (fig. 26). This tapers downward into the still normal stem 5–8 mm. below the cut surface. At the base of the 1 cm. segment, therefore, the cambium functions normally and xylem and phloem elements differentiate at the usual rate, while above, in the region of swelling, differentiation does not keep pace with the active division of the meristem cylinder cells. The daughter cells in this region are shorter and wider than normal and eventually differentiate as tracheids and shorter phloem elements.

Cell division is not confined to the central stele, but occurs also in the cortical bundles, phloem parenchyma, endodermis and pericycle, and occasionally in the adjacent cortical cells.

Rapidity of cell division is occasionally emphasized in the parenchyma tissues by the presence of 2–4-nucleate cells.

A very slight trace of a potential cork cambium is indicated in the outer cortex and pith in a few cells immediately below the cut surface, but it remains inactive.

The formation of lacunae in the swellings is not unusual. A break-

down of the middle lamella occurs in the parenchyma tissue, usually along the inner face of the cortical bundles. Lacunae begin to develop about the same time that primordia originate (fig. 27).

3. INITIATION AND DEVELOPMENT OF PRIMORDIA.—In the oldest seedlings (164 hours) the root primordia are apparent to the naked eye in serial sections, and may be demonstrated in the intact shoot by staining with IKI. As a rule they arise in whorls, one to four at any level, the oldest next to the cut surface, the younger progressively lower (figs. 9, 29). Initials appear in the rays, except in the region of maximum swelling, where origin, distribution, and number are apt to be somewhat less regular. In transverse section only one primordium usually appears in exactly median section, while the other two or three are tangentially cut. Thus orientation of rootlets at any one level is actually spiral, not cyclic as would appear from a superficial examination. Rootlets in general pass horizontally outward through the cortex, but occasionally they bend first downward, then outward.

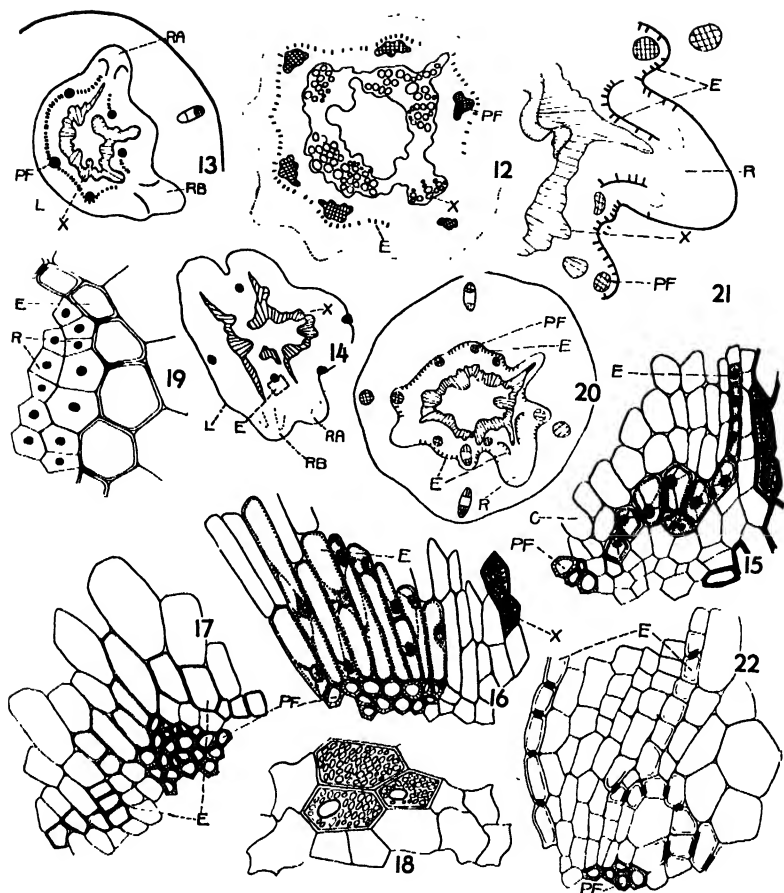
The development in time of the roots may be followed in material collected at stated intervals, and corroborated by the distribution in space of the primordia of different ages present in older swellings (116–164 hours). Origin and differentiation are intimately bound up with the formation of the meristem cylinder. In this connection sections of non-imbedded material stained with iodine are useful in following the distribution of the starch-containing cells. Near the lower limit of the older swellings, periclinal division is evident in the starch sheath cells, particularly across the ray (fig. 10). The plastids containing starch grains are distributed approximately equally to the daughter cells. Similar periclinal divisions appear next in the pericycle, and are followed immediately by anticlinal and irregular walls in both layers. Thereafter meristematic activity extends to the inner cortex. Root primordia are first distinguished as actively dividing cell groups, irregular in outline, near the outer margin of the rays. A primordium therefore is not distinctly traceable to pericycle alone, but arises in a complex of elements derived from inner cortex, endodermis, and pericycle.

Continued cell division results in the definition of a blunt root tip covered by an irregular sheath of starch containing cells (fig. 11), but the histogens are not defined at this stage. Elongation and dif-

ferentiation of the young root proceed in the usual way. Procambial strands give rise to stelar tissues, spiral vessels, and functional phloem elements. The latter develop in continuity with the axial phloem. Continuity between the water conducting tissues of axis and root is likewise established. Lignification of the ray cells at the base of the root gives rise to a central core of xylem, pitted tracheids, which connect laterally with the xylem of the axial vascular bundles.

In the lower internodes the change from typical starch sheath to typical endodermis, already described, is meanwhile effected. Demarcation of the endodermal region by lignification continues upward into the swelling. As might be expected, here the end result is somewhat different. The meristem cylinder, composite in origin, has differentiated by this time into cortex and stelar tissues. Intercellular spaces are absent in the stelar zone but abundant in the cortical, and are visibly blocked with auxin paste. (Since this cortical tissue originates from differentiation of the meristem cylinder, to clarify description it may be called meristematic, in contrast to normal primary tissue.) Differentiation of the endodermis, the boundary between stele and cortex, continues from the normal stem below, directly upward. Lignified Casparian strips differentiate on the walls of the innermost layer of this "meristematic" cortex. The development is gradual, so that in transverse sections at succeeding levels the endodermis appears as a complete or an interrupted ring (fig. 12). Lignification appears first across the ray arcs, and later across the pericyclic arcs. This lignification of the Casparian strip may be "typical," a narrow ring surrounding the cells, apparent therefore on the radial walls in transverse section; on the other hand it may be irregular in extent. Thus in transverse section the entire radial wall, and segments of the outer tangential wall, may react to phloroglucin and HCl. Occasionally an entire group of cells is affected, generally near the base of a root primordium (fig. 15). Intercellular spaces are always adjacent to lignified wall areas. The endodermis is interrupted by the developing roots, but because of its lignification, it may be followed from the axial cylinder outward, parallel with and external to the differentiating stele of the primordia. It is therefore in direct continuity with the differentiating endodermis of the young root (figs. 13, 15).

This appearance is strikingly different from that of an endodermis



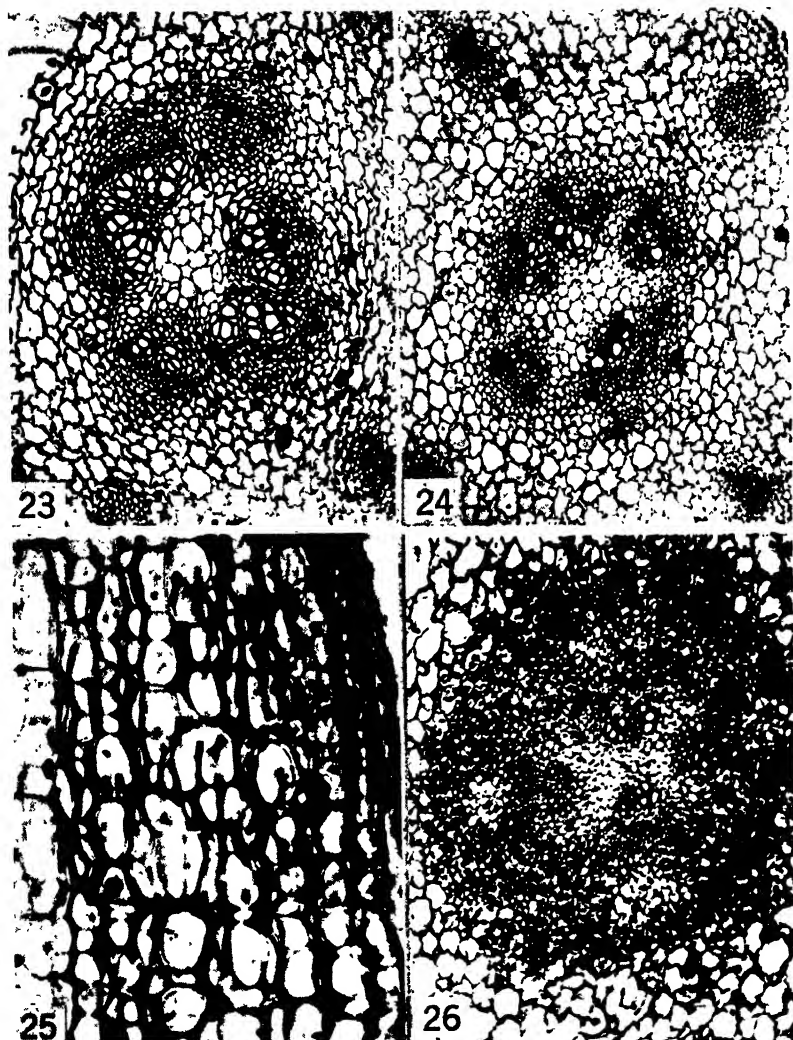
FIGS. 12-22.—Fig. 12, A.P. (164 hours) transection, base of swelling; endodermis not yet continuous. Fig. 13, A.P. (164 hours) transection, swelling with primordia *RA* and *RB*; axial endodermis continuous with developing endodermis of root *RA*; root *RB* cut tangentially. Fig. 14, A.P. (164 hours) transection, similar swelling cut at higher level; endodermis not yet present; two primordia *RA* and *RB*; for detail of region *E* see figs. 16 and 17. Fig. 15, A.P. (164 hours) transection at primordial base; axial endodermis continuous with developing endodermis of primordium; irregular lignification of “endodermal” group of cells (cf. fig. 13 root primordium *RA*). Fig. 16, A.P. (164 hours) detail of region *E*, fig. 14; radial elongation of endodermal cells. Fig. 17, A.P. (164 hours) transection near primordial base; radial elongation of endodermal cells near root base and irregular lignification in “endodermal” cell group. Fig. 18, A.P. (164 hours) lignification of pitted tracheids in ray and vessel-like perforations. Fig. 19, A.P.2. (160 hours) transection, detail of endodermis surrounding root primordium. Fig. 20, A.P.2. (160 hours) transection, primordium developing within endodermis. Fig. 21, A.P.2. (160 hours) detail of endodermis of axis and developing primordium of fig. 20. Fig. 22, A.P.2. further detail of same. (See footnote to figs. 1-11 for explanation of abbreviations.)

usually associated with root formation. In normal secondary root ontogeny the axial endodermis retains its identity, and by division and expansion keeps pace with the developing conical primordium. The endodermis of the young root meantime differentiates around the stelar cylinder and connects basally with the axial endodermal sheath. In the present instance the original starch sheath (or endodermis) is obliterated in cell division. A second functional endodermis appears after differentiation of the primordia and maintains continuity with the primary endodermis below.

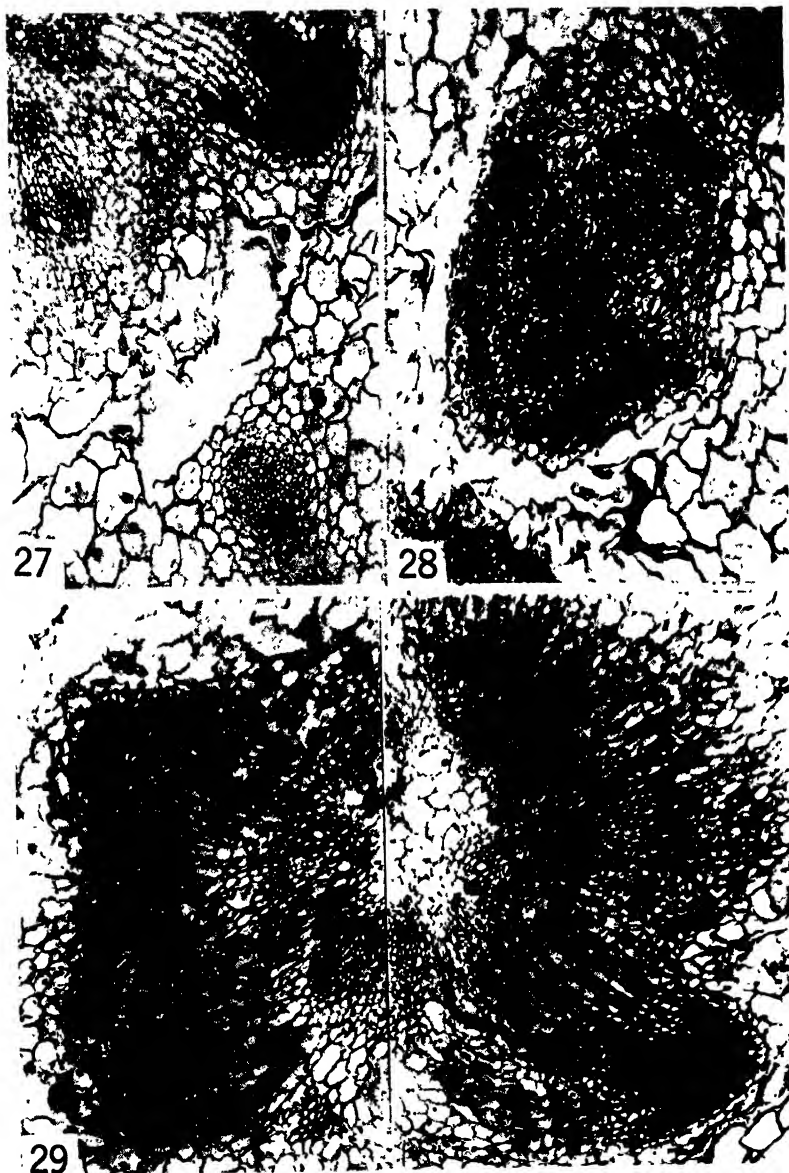
The endodermal cells usually are not exaggerated in size. Marked radial elongation, however, similar in appearance though not in ontogeny to that in *Phaseolus*, is occasionally observed. Root primordia may arise in adjacent rays at approximately the same level, and keep pace with each other in growth (fig. 14). Between two such bases lies a strand of primary pericyclic fibers and extra-pericyclic meristematic tissue, which from its position may be termed secondary endodermis. When the roots begin to lengthen rapidly, the secondary endodermal and adjacent cells expand in a radial direction to match the cells of the region of elongation of the young root (fig. 16). Endodermal cells farther removed from the root base may be similarly affected to a lesser degree. The radial walls of such cells may also become completely or partially lignified (fig. 17).

Root primordia originate also in connection with the cortical vascular bundles. Meristematic division begins in the internal phloem of the amphiphloic bundle, and in the adjacent endodermal and cortical cells the primordium with its root cap is gradually defined as before, and in this case is directed toward the center of the stem. During growth, however, it swings first tangentially and then vertically downward through the cortex. No primordia appear in connection with the cortical fiber strands, nor does cell division occur (fig. 28).

4. LIGNIFICATION.—Within the limited time of experimentation (164 hours) the lignification of tissues is not particularly evident. Lignification of the ray cells in the intervals between root primordia is somewhat sporadic. The resultant tracheids are approximately isodiametric, or slightly elongated. The wall marking is pitted or reticulate, and both types of marking are occasionally present on the



FIGS. 23-26.—Figs. 23, 24, difference in vascular xylem development in treated and control seedlings: 23, A.P. (164 hours) transection below swelling, 1 cm. below cut surface; 24, control decapitated stem at same level (164 hours). Fig. 25, A.P. (48 hours) longisection, penetration of auxin paste, stained with Sudan III. Fig. 26, A.P. (56 hours) transection, meristem cylinder.



FIGS. 27-29.—Fig. 27, A.P. (90 hours) transection, lacuna. Fig. 28, A.P. (164 hours) transection, cortical primordium. Fig. 29, A.P. (164 hours) transection near apex of swelling; three root primordia, only one approximately median. (See footnote to figs. 1-11 for explanation of abbreviations.)

same wall. Vessel-like perforations are commonly seen in transverse section. Lignification appears about 100 hours after auxin treatment, and is not observed in any control (fig. 18).

ANATOMY OF SWELLINGS (SERIES B)

The older internodes used in this series were decapitated and treated with auxin paste as before, and thereafter collected at intervals of 64, 112, and 160 hours. In the 64 and 112 hour specimens no root formation was indicated, and swelling in a few instances only was barely visible. Five of the sixteen seedlings allowed to grow for 160 hours developed small swellings and two to six roots. The swellings were generally split lengthwise along part of one side, and the root primordia were visible beneath or projected from the slit. The region of maximum swelling is 2 to 3 mm. from the cut surface and the tissue immediately adjacent to the latter 1 to 2 mm. is usually discolored and necrotic.

The chief points of comparison between this and the previous series are: (1) Meristematic activity is very much less in all tissues. (2) The parenchyma tissue is much less plastic, with the result that splitting of the cortex occurs during cell division and root formation. (3) The pith is very frequently entirely lignified. (4) The behavior of the endodermis forms the most interesting point of comparison (figs. 19-22).

As was expected, the Casparian strips of the endodermis were lignified in the lower internodes up to the base of the swelling. Within the latter the endodermis is more or less complete at successive levels. Whether it is unlignified or lignified as to Casparian strips, however, it maintains its identity as an individual layer, and is traceable around the developing root primordia (fig. 19). Periclinal cell division does not occur. As the primordium develops, a stelar endodermis is differentiated within it which connects basally with the axial endodermis, thus simulating the normal condition seen in secondary root development (figs. 20, 21, 22).

ANATOMY OF SWELLINGS (SERIES C)

The seedlings in this series were grown as in series A. When the leaf of the fourth node was apparent they were decapitated as

before, but the cut internodes were then inserted in aqueous solutions of auxin of varying concentration for forty-eight hours. Thereafter the seedlings were allowed to continue growth as before. The results are summarized in table 1.

It is seen from the table that the outline of the swelling and the number of roots depend upon the concentration of the auxin solution. The swelling varies in form from the blunt club seen in the weakest solution to the long ellipsoid ending in 2 cm. of shriveled necrotic axis, of the strongest solution (figs. 3, 4).

TABLE 1

CONCENTRATION IN MILLIGRAMS PER LITER	DISTANCE OF MAXIMUM SWELLING FROM CUT SURFACE (MM.)	LENGTH OF SWELLING (CM.)	NUMBER OF ROOTED SWELLINGS	NUMBER OF ROOTS PER SWELLING
100	1-2	1-1.5	4/10	1-13 roots 3-3 4 roots
200	2-3	1 7-2 5	10/10	All 12-18
400	4-5	2.5-3	10/10	All 60-90
1000	200	2.5-3	10/10	All 80-120

The anatomy of swelling and of root formation is essentially the same as in the paste series.

The meristem cylinder is differentiated as before, but the intercellular spaces are larger, resulting in a looser tissue. In the weakest solution meristem activity does not culminate in root formation. The intense development of roots seen in the strongest solution causes a vertical splitting of the stem. This region of the stem affected, 2 cm. below decapitation, has practically ceased to elongate and is therefore less plastic than the younger part of the internode.

The behavior of the endodermis is essentially similar to that in series A. As a starch sheath it becomes obliterated by intense meristem activity. By lignification of Casparian strips it is redefined in the region of the swelling.

From a comparison of series B and C it appears that the endodermis loses its capacity for division sooner than does the pericyclic and ray tissue.

Discussion

In one of the earliest surveys on root anatomy, JANCZEWSKI (3) outlines the various types of development seen in the primary and secondary roots of higher plants. The Leguminosae, including *Pisum sativum*, differ from the majority of other dicotyledons, in that secondary roots originate from three cell layers (pericycle, endodermis, and inner cortex) and the histogens are differentiated comparatively late (current terminology is used here in place of the terms pericambium, etc.).

VAN TIEGHEM and DOULIOT (6) later rejected JANCZEWSKI's findings in reference to the Leguminosae, and attributed the entire primordium of the secondary root to division of pericyclic cells. A temporary digestive pocket or sheath only, originates from the outer tissues, the endodermis, and the cortex. Adventitious roots, arising from a hypocotyl base, are essentially similar in origin to secondary roots. The primordia of adventitious and secondary roots, while both endogenous, necessarily vary somewhat in their method of differentiation. In the present instance, where etiolation induces the formation of a root endodermis in the stem, the situation becomes more complex.

The root primordia of *Pisum* vary in origin according to the age of the internode and the concomitant development of the endodermis. In the younger seedlings (series A) the primordium is derived from a complex of tissues—endodermis, inner cortex, and pericycle. It thus resembles the original description given by JANCZEWSKI. In the older specimens (series B), whether or not the Casparian strip is lignified, the endodermis maintains its identity. The primordium is therefore derived from the pericycle as VAN TIEGHEM has indicated. No comparison of JANCZEWSKI's and of VAN TIEGHEM's accounts of root initiation in the normal plants has been made at the present time.

In recent papers KRAUS (4) and BORTHWICK (1) and their collaborators trace the root primordia to a complex group of meristem cells, not to a single definite layer. The younger seedlings of *Pisum*

therefore resemble fundamentally the seedlings of *Phaseolus* and *Solanum*.

Certain differences are observed, however, in the specific tissue reactions of the three genera. The endodermis of *Phaseolus*, for instance, "is highly responsive to indoleacetic acid. Within 168 hours, cells 5 cm. below the point of application are highly meristematic" (4). In etiolated seedlings of *Pisum* the stimulus to active cell division does not in any case extend below 6–8 mm. in 164 hours. There is thus a marked difference in the reaction of the endodermis cells in the two genera. Whether this reaction is specific or is conditioned by the etiolation of *Pisum* is undetermined.

Another instance of marked difference in activity is seen in the xylem parenchyma and in the pith. In *Phaseolus* both these tissues become meristematic, whereas in *Pisum* the pith cells are inactive and the xylem parenchyma practically so.

The positions of the primordia are also contrasted. Whereas in *Pisum* the root initials occur most commonly in the rays, in *Phaseolus* and also in *Solanum* they appear in the phloem. In *Solanum* a double ring of root primordia is developed in definite relation to the phloem strands of the bicollateral bundles.

It is clear therefore that the general reactions of living stem tissues to auxin so far established, namely, cell expansion, meristematic activity, tumor formation, and root development, occur in *Pisum* as in other seedlings previously described. Differences in detail appear in the specific reactions of individual tissues.

Two general conditions essential for the initiation of meristem activity are noted by PRIESTLEY (5): the presence of a blocking surface and a source of food material. The penetration of lanolin (control) and lanolin-auxin paste (experimental) into the cortical intercellular spaces suggests such a possible barrier, and a food supply is available in the adjacent phloem. The penetration of lanolin alone, however, does not induce cell division or swelling.

Attention has been called to the transformation, during etiolation, of the limiting layer of the cortex from a starch sheath to an endodermis with characteristic Casparian strips. An endodermis is structurally a barrier layer. Since in series A lignification of the Casparian strip does not precede differentiation of the root primordia, the

endodermis does not function in this connection. In series B, on the other hand, the primordial development occurs within the endodermis, as in the "typical" origination of a secondary root.

The vertical distribution of the root primordia has been commented upon. There is no obvious anatomical differentiation to account for their initiation. No unusual condition of intercellular spaces, no variation in lanolin infiltration, no change in endodermal structure is detectable when and where the root initials are first distinguishable. The appearance of primordia at definite vertical intervals indicates a periodic recurrence of the conditions and factors necessary for root formation. The solution of this problem depends on further physiological experimentation.

There is a lack of specificity of tissues. A generalized meristem cylinder arises from division of inner cortex, endodermis, pericycle, and other cells. From this apparently homogeneous tissue there are later differentiated such distinct elements as (1) endodermal cells with Casparian strips in a continuous cylinder one cell thick; (2) characteristic pitted tracheids, approximately isodiametric, in rays, root bases, and sporadically in inner cortex; and (3) parenchymatous elements of inner cortex and phloem.

Summary

1. Three series of etiolated seedlings of *Pisum sativum* were grown, decapitated in the third internode, and treated with auxin paste or with aqueous solutions of auxin. The treated seedlings were collected at eight hour intervals and observed for 164 hours. Controls were grown under exactly similar conditions.

2. The general results of auxin treatment, swelling and root formation, vary in extent in relation to seedling age and auxin concentration.

3. The form of the swelling varies in reference to auxin concentration.

4. Four phases of growth are recognized during the formation of a swelling, each characterized by a dominant activity: (a) expansion of parenchyma cells and infiltration of auxin paste into intercellular spaces (0-48 hours); (b) formation of a meristem cylinder from division of inner cortex, starch sheath, pericycle, and phloem paren-

chyma tissue (16-72 hours); (c) initiation and elongation of root primordia (56-164 hours); (d) lignification of tissues (116-164 hours).

5. The root primordia vary in origin in younger and older stems. In younger seedlings the identity of the endodermis is lost in the meristem cylinder complex. The root primordia are traceable to a group of ray cells within this complex. In older seedlings the endodermal cells do not divide and the primordia are therefore pericyclic and intra-pericyclic in origin. The endodermis is traceable as a primordial sheath. Casparian strips may or may not be lignified.

6. The roots arise approximately in whorls at successive levels on the stem. There is no visible anatomical differentiation to account for this distribution.

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GROWTH AND TROPIC RESPONSES OF EXCISED AVENA COLEOPTILES IN CULTURE¹

GEORGE S. AVERY, JR., AND CARL D. LA RUE

(WITH SIX FIGURES)

Introduction

If the *Avena* coleoptile is removed from the seedling and transferred to a suitable culture medium, to what extent is it able to grow and utilize food and growth hormone artificially supplied to it? To what extent does it possess reserves of food and growth hormone at the time of excision? Is growth hormone really necessary for growth?

Most previous experiments involving growth of the *Avena* coleoptile and its response to various stimuli have been done with the intact seedling, and from this work have come two views: (1) that the tip of the coleoptile is the center of hormone synthesis in the seedling; and (2) that when this tip is decapitated, the upper end of the coleoptile stump, after approximately 2.5 to 3 hours, begins to function as a new physiological tip, producing growth hormone and enabling the stump to renew its growth.

Evidence is gradually accumulating that these views are in need of revision. For example, SÖDING (8) discovered that physiological regeneration took place to an equal extent whether one or more millimeters of the coleoptile tip were removed. This suggests that a precursor or even the auxin itself must be coming from another part of the seedling. POHL (6) reported that the coleoptile tip was not the center of hormone synthesis, but that it dispersed substances conveyed to it from the endosperm. POHL also added auxin-a to the seed; it was transported to the coleoptile where it stimulated growth. HEYN (3) found that in cut-off cylinders (segments) phys-

¹ Contribution from the Department of Botany, Connecticut College, and no. 664, Papers of the Department of Botany, University of Michigan. A part of this work was done at the University of Michigan Biological Station.

iological regeneration does not occur; however, when BONNER (2) immersed such cylinders in solutions of varying concentrations of growth hormone, growth was promoted at lower but retarded at higher concentrations.

The work of JOST and REISS (4, 5) has shown that segments 20 mm. in length (cut from coleoptiles 2-4 cm. long) undergo appreciable elongation in the first twenty-four hours after excision, the extent of elongation depending upon the concentration of indole(3)-acetic acid in the culture solution, and whether the tip or basal end was immersed. From the work of SÖDING, and POHL in particular, it seems clear that in the normal seedling the growth hormone, or a precursor, moves upward from the endosperm and cotyledon through the vascular system to the tip of the coleoptile. From the tip it is dispersed downward through the coleoptilar tissues, presumably stimulating growth as it goes.

In the experiments reported here we have excised the young coleoptiles from their seedlings, thus removing their normal supply of food, water, minerals, etc., and cultured them in various ways.

Material and methods

Seeds of *Avena sativa* var. Victory (Svalov) were disinfected in a solution of calcium hypochlorite, transferred to sterile petri dishes containing moist filter paper, and germinated at 20°-25° C. When the coleoptiles were 3-12 mm. in length, they were excised from the developing seedlings and grown in both agar (under aseptic conditions) and liquid cultures (fig. 1). Certain cultures were supplied with sucrose or fructose, White's nutrient solution but without yeast (10), and indole(3)acetic acid; others were grown on pure agar or in distilled water. All cultures were grown in darkness at 20°-22° or 25°-26° C., and examined only in phototropically inactive light. Certain excised coleoptiles growing in culture were decapitated and then capped with agar blocks containing various combinations of mineral nutrients, sucrose, and indole(3)acetic acid; others were grown in an inverted position, with the decapitated distal end immersed in the solution (experiments without success on coleoptiles of the ages used).

Investigation

GROWTH HORMONE CONCENTRATIONS IN TIPS OF COLEOPTILES OF DIFFERENT AGES, FROM NORMALLY GROWING SEEDLINGS

One to 1.5 mm. tips of coleoptiles 2-47 mm. in length were removed, passed through a thin film of 5 per cent gelatin on a glass slide, and applied unilaterally to decapitated test coleoptiles. The first foliage leaf had not yet burst through the tips of any of the oldest coleoptiles tested. The results are plotted in figure 2. The

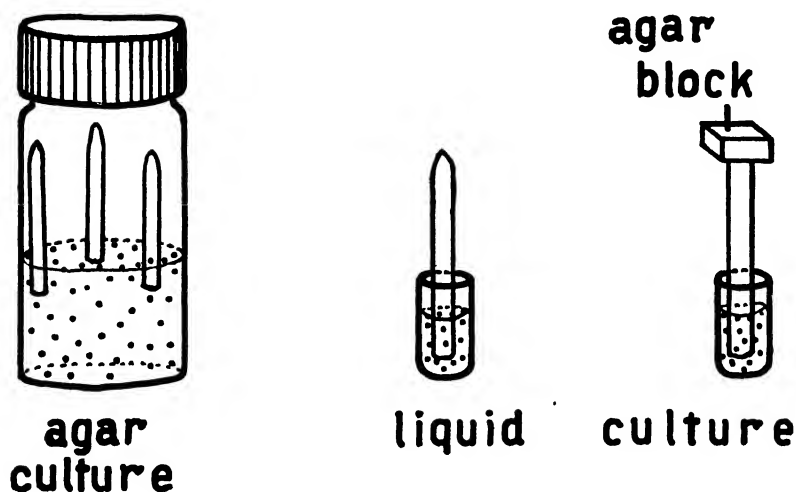


FIG. 1. —Methods of culture. The small glass containers used for liquid culture were supported in racks of 12 each. See table 2 in reference to figure on right.

tips of very young coleoptiles contained relatively low concentrations of growth hormone (see also WENT and THIMANN, 9). The most rapid increase in concentration occurred between the 2 and 6 mm. stages; this is correlated with the peak period of cell multiplication (1). No further appreciable increase was detectable after the coleoptile was 1 cm. in length although a peak content occurred near the end of the growth period.

From the preceding it is evident that tips from coleoptiles 3 mm. or less in length, excised for growth in culture, contain approximately one-half the hormone present in tips from coleoptiles 5-6 mm. or larger. If the hormone is the limiting factor for the growth of excised coleoptiles, those 6 mm. or more in length at the time of

excision should undergo a proportionately greater increase in length than those of approximately 3 mm. length, provided other factors are not limiting. The experiments which follow show this not to be the case.

GROWTH OF EXCISED COLEOPTILE AS AN INDEPENDENT ORGAN IN AGAR CULTURE

INFLUENCE OF SIZE OF COLEOPTILE AT TIME OF EXCISION, AND OF VARIOUS COMBINATIONS OF NUTRIENT MATERIALS. Two principal sizes of coleoptiles were used in this experiment in order to determine: (1) relationship of hormone content of tip at the time of excision to later growth of coleoptiles in culture; and (2) relationship between number of cells present at the time of excision (that is, the beginning reserve) and subsequent growth in length of excised coleoptiles.

Comparison of figures 2 and 3 indicates what at first glance appears to be a direct correlation between hormone content of tip and subsequent growth in culture. Comparison of percentage increase in length, however, reveals that coleoptiles in the series 2.6 to 2.7 mm. long at the time of excision, and with full nutrition, increased in length 434 per cent in the eight day culture period, whereas those with similar nutrition in the series 5.1 to 5.4 mm. at the start increased only 243 per cent. Similar relationships hold in the other series. Actually therefore there is an inverse relationship between hormone content of the tip at the time of excision and subsequent growth of the coleoptiles in culture. Such evidence suggests the view that growth hormone is not limiting for growth of coleoptiles in culture.

It has been shown previously that cell division goes on rapidly in the parenchyma of young coleoptiles (1): the number of cells in the subepidermal layer (median longisection) of a 2.5 mm. coleoptile approximates 90, that of a 5.0 mm. coleoptile, about 145. The growth curves of the coleoptiles in the series 2.71 mm. at the beginning of the experiment (fig. 3), grown on agar with White's solution, fail to attain the final length of those with similar nutrition of 5.3 mm. average length at the beginning of the experiment. With no further increase in numbers of cells, elongation of the cells already

existing at the time of excision (90 and 145 respectively) would account for the differences in final length. Cell division apparently did not continue under these culture conditions. However, even under such conditions of nutrition with sucrose lacking, the shorter excised coleoptiles continued to elongate for five days in culture.

The presence of sucrose in the nutrient medium enabled coleoptile growth to continue for seven and eight days, but again the coleoptiles which were short at the beginning of the experiment never attained the final length of those which were longer at the beginning, although

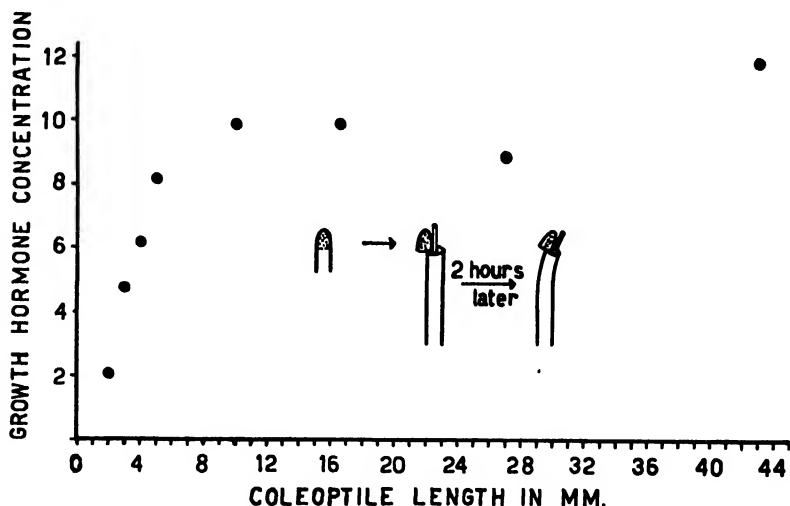


FIG. 2.—Concentrations of growth hormone in coleoptile tips of normal seedlings of different ages. Each point represents average of 48 coleoptile tips tested. Seedlings grown and tips tested in darkroom at 26° C. and 88–90 % relative humidity.

their percentage increase in length was much greater for agar and sucrose, 404 against 267 per cent; full nutrition, 434 against 243 per cent. A few cell counts in longisections of the coleoptiles showed a greater proportional increase in the number of cells in the short set than in the long (final average cell length was approximately the same in both sets). Hence, with sucrose in the culture medium, short coleoptiles with less reserve make a proportionately greater growth by adding appreciably to their reserve.

To determine further the effect of nutrition on subsequent growth of coleoptiles of different ages at the time of excision, three addi-

tional series of experiments were set up (fig. 4). Growth of these older coleoptiles was substantially completed at the end of four days in culture. Hence the older the coleoptile at the time of excision, the less the growth response in culture.

INFLUENCE OF INDOLE(3)ACETIC ACID SUPPLIED THROUGH CULTURE MEDIUM. Six series of coleoptiles, thirty-six in each series, were excised from seedlings when they had reached a length of 4 mm.

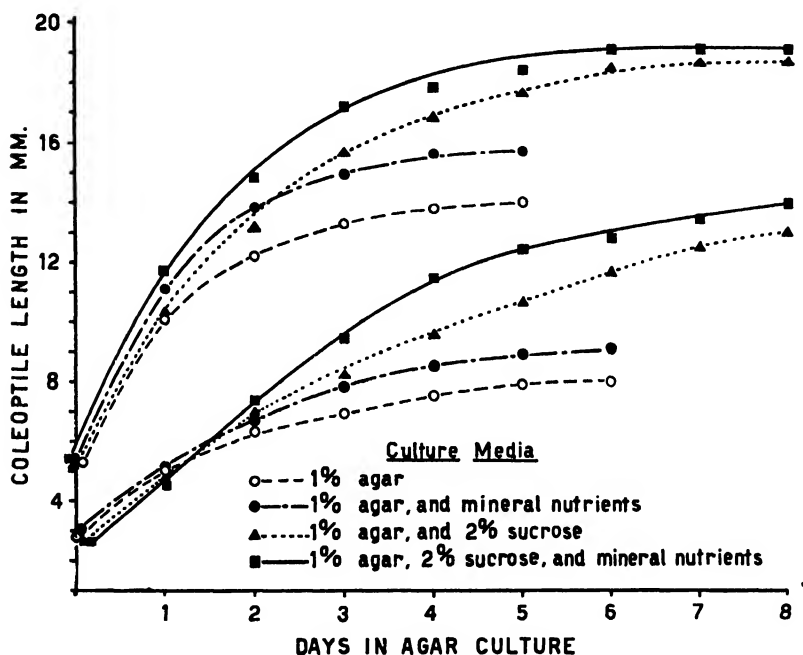


FIG. 3.—Growth of coleoptiles in agar culture with varying conditions of nutrition. Coleoptiles were of two different age groups at time of excision: 2.6–2.7 mm. and 5.1–5.4 mm. in length. There were 12 coleoptiles in each series. Darkroom temperature, 20°–22° C. See fig. 1 for method of culture.

Their cut bases were inserted into 1.5 per cent agar containing mineral nutrients, 2 per cent sucrose, and different concentrations of indole(3)acetic acid. They were allowed to grow for eight days in a darkroom at 24° C. The results are shown in figure 5.

In all the concentrations tested, indole(3)acetic acid showed a depressing effect on growth in length. This effect was noticeable in

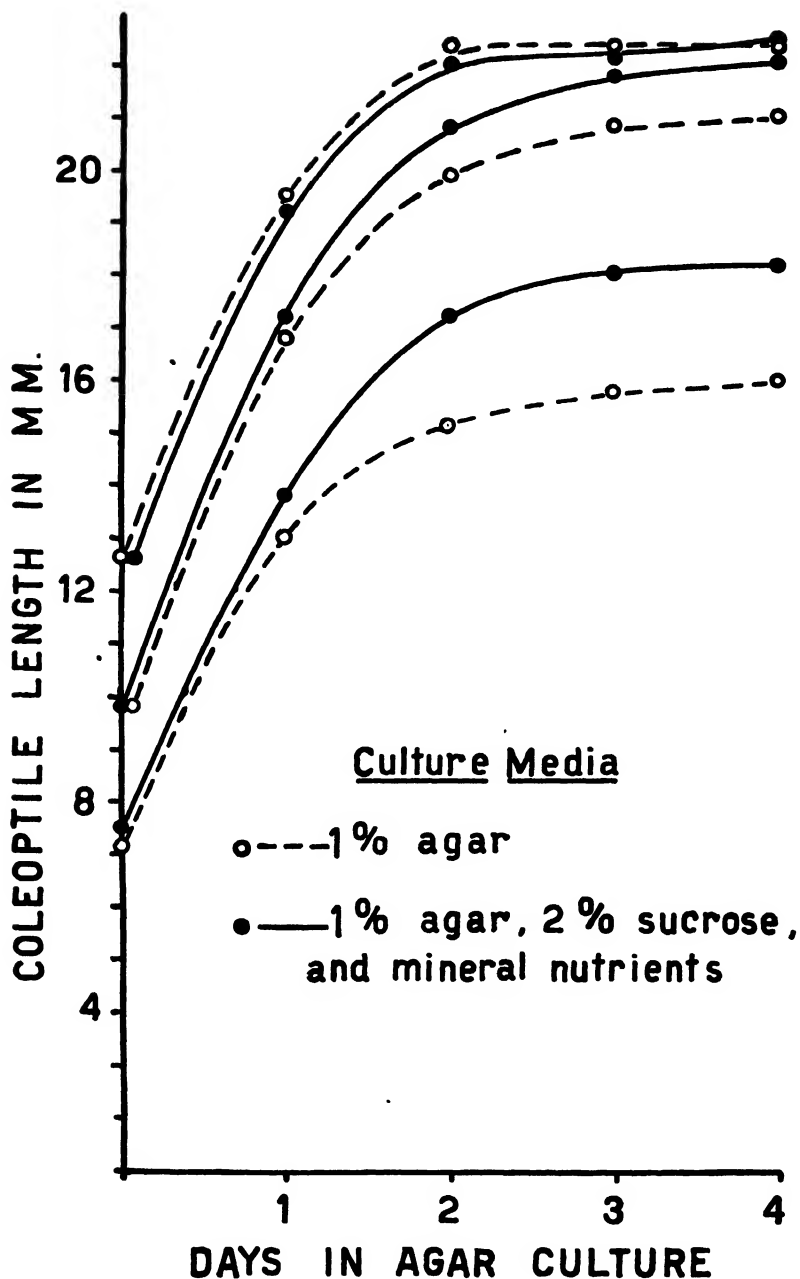


FIG. 4. --Continuation of fig. 3. Coleoptiles were of three different age groups at time of excision. Note influence of size of coleoptile at time of excision upon subsequent response to nutrition during growth in culture. Darkroom temperature, 26° C.

dilutions as great as 1:5,000,000, and higher concentrations retarded growth to correspondingly greater degrees. Computations show that the apparent differences in growth shown in figure 5 are statistically significant. Is it possible that physiologically similar substances produced in the normal seedling have no causal effect on growth in length of the coleoptile?

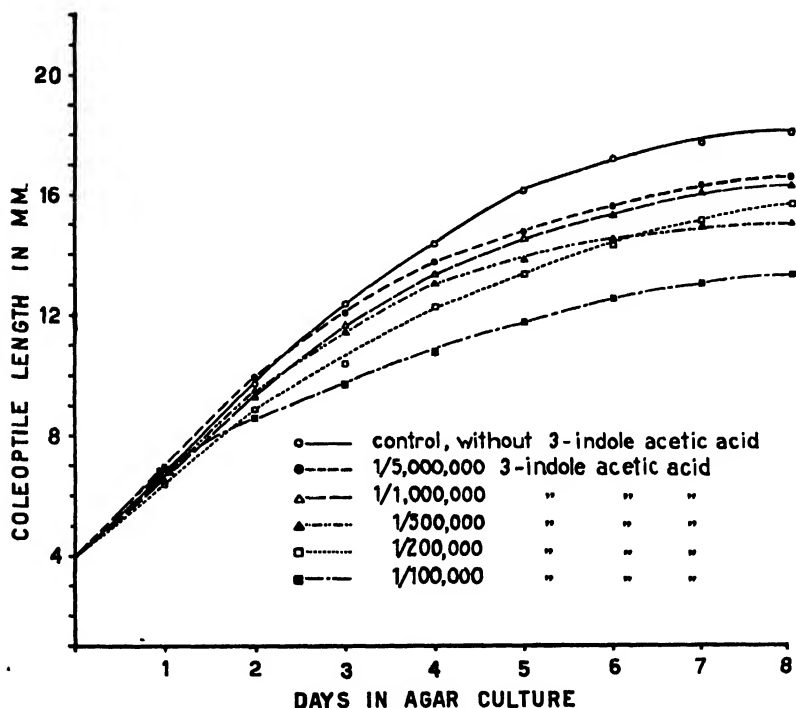


FIG. 5.—Influence of indole(3)acetic acid (supplied through culture medium) upon growth of coleoptiles in culture. Darkroom temperature, 24 °C.

The lowest concentration of indole(3)acetic acid used in this experiment (1/5,000,000) produces about 15° curvature when applied unilaterally to decapitated coleoptiles. WENT and THIMANN (9) recorded approximately 21° curvature for the same concentration. The substance which produces curvature proportional to concentration when applied to the tips of decapitated seedlings also causes depression of growth proportional to concentration when applied at the bases of excised coleoptiles.

INFLUENCE OF pH OF CULTURE MEDIUM. --Experiments involving the growth of excised coleoptiles on agar adjusted with hydrochloric acid (not buffered) to pH 4, 5, 6, and 7 yielded no appreciable nor constant differences.

GROWTH OF EXCISED COLEOPTILE AS AN INDEPENDENT ORGAN IN LIQUID CULTURE

In order to make certain that an adequate supply of water was available to the artificially cultured coleoptiles, liquid cultures were set up. The base of each coleoptile was placed in a small glass culture cup, as illustrated in figure 1, and the solutions were changed daily at the time the coleoptiles were measured. In order to be certain that liquids could enter the coleoptiles readily, about 0.2 mm. was cut from the base of each at the time of changing the culture solution. To test the entrance of liquids, forty-eight excised coleoptiles were placed in culture cups containing dilute solutions of fast green, green ink, etc. In every instance the dye had ascended to the tip of the coleoptile within a two hour period, thus leaving no doubt that materials present in the culture solution were being taken up by the coleoptiles.

INFLUENCE OF SUCROSE IN CULTURE SOLUTION.—In order to determine whether certain concentrations of sucrose might effectively promote growth of older excised coleoptiles in liquid culture, culture solutions containing 2 to 10 per cent sucrose were set up (table 1). The coleoptiles were 9.5 to 12.5 mm. in length (in agar culture, 2 per cent sucrose had a slightly favorable effect on subsequent growth of coleoptiles excised when approximately 10 mm. in length), and therefore well past the main period of cell division at the time they were excised. Growth of the coleoptiles in the lower concentrations of sucrose (2 to 4 per cent), although not so good as the controls over the first twenty-four hours, gave closely parallel results; successively higher concentrations resulted in proportionate decreases in growth. However, higher concentrations of sucrose extended the length of the growth period by one to two days, and gave somewhat better growth on the fourth and fifth days. Similar experiments, using fructose, yielded results of the same order. Hence the conclusion is that in liquid culture, as in agar culture, the addition

of sugar to the culture medium was not advantageous to the growth of older coleoptiles.

INFLUENCE OF CAPPING DECAPITATED COLEOPTILES WITH AGAR BLOCKS CONTAINING VARIOUS NUTRIENTS AND INDOLE(3)ACETIC ACID.—In order to test the influence on growth of agar blocks containing White's solution with and without sucrose, coleoptiles again were

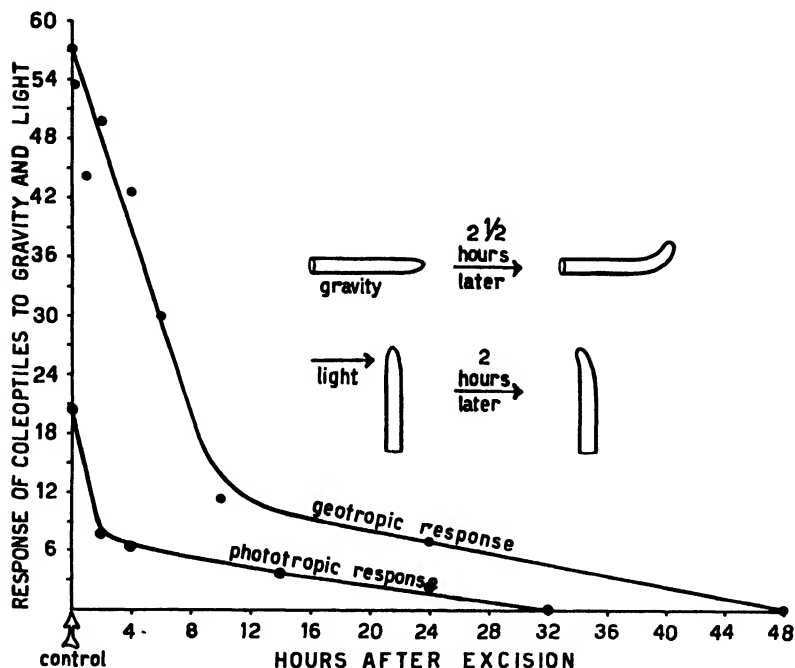


FIG. 6.—Geotropic and phototropic response of excised coleoptiles. Each point on the graph represents average of 12 coleoptiles. Coleoptiles were kept in an upright position (from time of excision through period of stimulation) on moist filter paper in vertically placed petri dishes. Geotropic stimulation for $2\frac{1}{2}$ hours before recording curvature. Unilateral light stimulus from 40-watt Mazda lamp at a distance of 15 feet for 2 minutes; curvature recorded 2 hours later.

used which were past the cell division phase of their development; they were 12.5–13.7 mm. in length after decapitation, at the time of placing in culture cups in distilled water. The agar blocks were placed on the apices of the decapitated coleoptiles (fig. 1). There were no appreciable differences in growth, whether the coleoptiles were capped with blocks made up with distilled water only, or with White's solution, or White's solution with sucrose or fructose.

In order to determine the influence on growth of agar blocks containing indole(3)acetic acid, coleoptiles 18–19 mm. in length were excised, placed in culture cups in distilled water, and decapitated to

TABLE 1

GROWTH OF EXCISED AVENA COLEOPTILES IN LIQUID CULTURE WITH VARYING CONCENTRATIONS OF SUCROSE. TWELVE COLEOPTILES IN EACH SET, RANGING IN LENGTH FROM 9.5 TO 12.5 MM. AT BEGINNING OF EXPERIMENT. DARK-ROOM TEMPERATURE 26° C.; RELATIVE HUMIDITY 88–90 PER CENT

CULTURE MEDIUM	DAILY GROWTH INCREMENT IN PERCENTAGE OF PREVIOUS DAY'S LENGTH					
	1	2	3	4	5	6
Distilled water.....	61	12	7	3	0	0
Sucrose 2%.....	48	13	9	2	0 5	0
3%.....	46	10	4	2	0
4%.....	46	11	10	1	2	0
5%.....	35	14	13	9	3	0.5
6%.....	25	11	9	6	2	0.5
7%*.....	19	5	4	2	0.5
8%.....	18	11	4	5	4	0
9%.....	16	11	7	10	4	0
10%.....	13	14	6	7	2	0

* Growth continues through 9th day in cultures with 7–10 per cent sucrose; best growth in 8–9 per cent sucrose.

TABLE 2

INFLUENCE OF INDOLE(3)ACETIC ACID ON GROWTH IN LENGTH OF AVENA COLEOPTILES; APPLIED IN 1.5 PER CENT AGAR BLOCKS TO DISTAL CUT SURFACE OF COLEOPTILE STUMP. RESULTS GIVEN IN PERCENTAGE INCREASE OVER CONTROLS (PURE AGAR 1.5 PER CENT) AFTER 24 HOURS. TWELVE COLEOPTILES IN EACH SET. DARKROOM TEMPERATURE 26° C.; RELATIVE HUMIDITY 88–90 PER CENT

COMPOUND	CONCENTRATION			
	Dilution: 1:3,200,000 γ per liter: 312	1:800,000 1250	1:20,000 50,000	1:5,000 200,000
Indole(3)acetic acid ..	10.4	13.4	18.3	13.4

16 mm. in length. The agar blocks were placed on the apices of the decapitated coleoptiles, with resulting elongation appreciably greater than the controls (table 2).

For straight growth of coleoptilar stumps still attached to their

seedlings but with indole(3)acetic acid as applied in this experiment, SCHEER (7) found a 31.9 and 25.9 per cent increase in length over the controls for 1:20,000 and 1:5000 respectively.

GEOTROPIC AND PHOTOTROPIC RESPONSE OF EXCISED COLEOPTILES

That excision has a marked effect on the capacity of coleoptiles to respond to the stimuli of gravity and light is clear from figure 6; no attempt was made to adjust the periods of stimulation so that the initial magnitude of curvature would be the same. In the phototropic experiment the coleoptiles were 20 mm. long at the time of excision, while in the geotropic experiment they ranged from 30 to 35 mm. in length. The capacity to respond fell to approximately 20 per cent of that of the controls in 10–14 hours after excision. Contrasted with this is a drop to 55–65 per cent (of that of the intact coleoptiles) in the output of growth hormone by the tips of such excised coleoptiles after twelve hours' excision.

Summary and conclusions

1. Determinations were made of the hormone concentration in the tips of normal coleoptiles from seedlings of different ages; the tips of very young coleoptiles contain relatively low concentrations and the most rapid increase in concentration occurs while coleoptiles elongate from 2 to 6 mm. After the coleoptile is 1 cm. long, hormone content in the tip remains at a relatively constant level on to the end of the growth period.

2. Coleoptiles of different ages were excised and grown aseptically on 1 to 1.5 per cent agar, agar with sucrose, and agar with sucrose and mineral nutrients; indole(3)acetic acid was supplied through the culture medium in certain experiments. Coleoptiles were grown also in liquid cultures. The coleoptiles grew equally well on agar adjusted (not buffered) to pH 4, 5, 6, or 7.

3. Coleoptiles excised at a length of 3 mm. or less failed to reach the length attained by coleoptiles grown under the same conditions which were more than 5 mm. long when excised. However, the 3 mm. coleoptiles showed a far greater percentage increase in length than those 5 mm. long at the start of the experiment (although the initial hormone concentration in the tip was much lower). The addi-

tion of sucrose and mineral nutrients had a markedly beneficial effect on growth of such coleoptiles in culture; when 12 mm. or more in length, nutrient substrate had no effect on growth in agar culture. Hence the older the coleoptile at the time of excision, the less the growth response in culture.

4. Low concentrations of sucrose in the liquid culture medium gave better results than high, although high concentrations prolonged the growth period somewhat.

5. The addition of indole(3)acetic acid to the nutrient agar in which excised coleoptiles were grown retarded growth in length. This retardation was proportional to the concentration of the indole(3)acetic acid.

6. Decapitated excised coleoptiles capped with agar blocks containing indole(3)acetic acid showed significant length increases over the controls.

7. Excised coleoptiles gave geotropic and phototropic responses of rapidly decreasing magnitude over a twenty-four hour period. The capacity to respond fell to approximately 20 per cent of that of the controls ten to fourteen hours after excision; meanwhile hormone concentration in the coleoptile tips fell only to 55-65 per cent of that of the controls.

8. In conclusion, it is clear: (1) That growth hormone secreted by the coleoptile tip (as judged by concentration in the tips of young and older coleoptiles) has no helpful relationship to growth of coleoptiles in culture. The smaller the coleoptiles at the time of excision the greater their percentage increase in length in culture, in spite of their relatively lower content of hormone at the time of excision. (2) That growth of coleoptiles excised when approximately 3 mm. in length continues in agar culture (at 20°-22° C. and with suitable nutrition) for a period of eight days, or more.

9. In no instance, with present methods, have we found detectable concentrations of growth hormone in the tips of coleoptiles more than forty-eight hours after excision. If the substance is truly a necessity for coleoptile growth, the concentrations at which coleoptile elongation is stimulated are very very low, and growth must continue for six days at such undetectably low concentrations. The evidence makes another explanation equally plausible—that growth

hormone is not a necessity for elongation of the coleoptile. Such an interpretation would not mean that growth hormone cannot stimulate growth of the coleoptile, for indeed it can, as illustrated throughout the voluminous literature on this subject, and shown again in table 2 of this paper. But nowhere has it been shown beyond question, in our judgment, that the hormone must be present if growth in length is to occur. Hence we suggest here a probable difference between growth stimulating effects of such physiologically active substances and their necessity for length growth. We regard as doubtful the view that growth hormone is a necessity for growth; that it can catalyze growth is quite another matter.

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RELATION OF ENVIRONMENT AND OF THE PHYSICAL PROPERTIES OF SYNTHETIC GROWTH SUBSTANCES TO THE GROWTH REACTION

DAVID M. BONNER

(WITH SIX FIGURES)

Introduction

A molecule must possess at least two general characteristics to function as an auxin or plant growth hormone. In the first place it must be transportable within the tissues of the plant; in the second place it must be capable of taking part in the chain of processes leading to cell elongation. In the pea test (10) longitudinal transport plays no part, and hence it is possible with the aid of this test to study the primary growth activity (8) of an auxin. A more detailed investigation of the reactions of a number of auxins in the pea test has been carried out, and is reported in this paper.

It was noted by KÖGL and HAAGEN SMIT (4, 5) while isolating pure auxin, that in testing the extracts in the *Avena* test it was of advantage to add small amounts of potassium chloride, and of acid. DOLK and THIMANN (2) investigated this same phenomenon while studying the nature of the growth substance, indole(3)acetic acid, produced by *Rhizopus sinuus*. This substance, in a concentration which showed slight activity in the *Avena* test when buffered at pH 7.0, was highly active when buffered at pH 5.0. These are apparently the first experiments in the field of plant hormones demonstrating the effect of the pH of the external solution on the activity of a growth promoting substance. Since that time (1931-32) there have appeared numerous papers dealing with this subject. One main hypothesis has developed as to this effect of acid on growth. J. BONNER (1), working with the *Avena* coleoptile, obtained a curve closely resembling the dissociation curve of a weak acid by plotting degrees acid curvature in his *Avena* test against the pH of the external solution. From this and further data he postulated that the action of acid on growth is through its direct action on auxin; and that auxin,

an organic acid, was active only in the non-dissociated state. A study of growth substances with different activities possessing different pKs (the negative logarithm of the dissociation constant) should thus give an insight into whether the difference in activities is due to a change in the essential molecular structure, or due only to a difference in physical properties.

Methods

The method of assay used was the pea test as formulated by VAN OVERBEEK and WENT (9). The indole(3)acetic acid (melting point 164° C.) used was kindly supplied by Merck, and the other growth substances used were either synthesized or purified by recrystallization by Dr. J. B. KOEPFLI. All pH measurements were made electrometrically, a glass electrode being used in a majority of the measurements. The buffer solutions were mixtures of (1) disodium hydrogen phosphate and potassium dihydrogen phosphate, (2) disodium hydrogen phosphate and citric acid,¹ or (3) potassium acid phthalate and either hydrochloric acid or sodium hydroxide. The particular buffer mixture used played no role in the activities obtained, as determined experimentally not only by the writer but also by other workers in this laboratory. The concentration of the buffer solution used was approximately 0.01 molar, but was non-toxic within a tenfold range of concentration.

Investigation

NATURE OF THE ACID CURVATURE

In order to gain a better understanding of the data, it was found necessary to determine first of all the nature of the acid curvature. This curvature is a phenomenon observed when split pea sections are placed in solutions of an acid pH. The sections show a strong backward curvature (fig. 1). VAN OVERBEEK and WENT (9) have shown that the growth curvature is due to the faster growth rate of the side with the intact epidermis in comparison with the cut surface. The acid curvature must therefore be due to the more rapid growth of the cut surface than of the intact surface. This would easily be explain-

¹ The citric acid used was of sufficient purity that growth curvatures as noted by VAN OVERBEEK and WENT (9) were not found.

able, on the basis of J. BONNER's (1) work, as being due to the ability of the buffer to penetrate from the cut surface. In the case of penetration of an acid buffer, a lowering of the internal pH on the cut surface would result, causing the formation of active non-dissociated auxin from the inactive dissociated auxin present.

There are two obvious means of testing this hypothesis: (1) measuring the growth in length of sections with and without epidermis immersed in buffer solutions of varying pH, and (2) determining the change in internal pH of sections with and without the epidermis when placed in solutions of varying pH. Data from such experi-

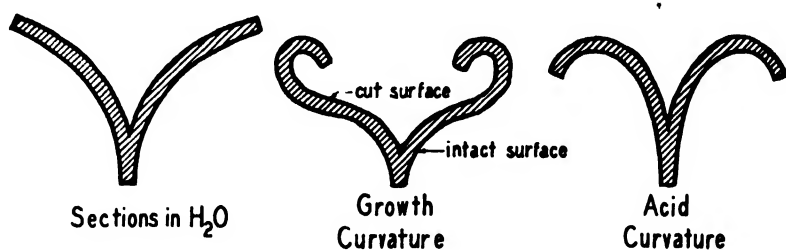


FIG. 1. The pea test. Left: section of stem of etiolated pea seedling split longitudinally and placed in water. Center: similar section after treatment with solution containing an active auxin. Right: similar section after treatment with an acid solution containing no auxin.

ments are presented in tables 1 and 2. From these tables it is clear that: (1) Sections without the epidermis grow more rapidly at a pH of 4.0 than do intact sections at the same pH. At a pH of 7.0, however, the growth of the two is about the same. (2) Split sections when placed in a buffer solution of pH 4.0 show a change in internal pH corresponding to an increase of 2.7 times in amount of non-dissociated auxin present (from 3 to 8 per cent non-dissociation). The internal pH of intact sections on the other hand show no appreciable change.

The pH of the split sections was determined by splitting sections as for the regular pea test and floating them on the solutions for twenty-four hours. The split portions were then cut away from the solid base, ground in a mortar, the expressed sap decanted into the electrode vessel, and the pH determined with a glass electrode. The time between grinding of the sections and the initial pH determination was approximately one minute. Table 2 shows that this de-

crease in pH makes more auxin available for growth on the cut surface, giving rise to a growth gradient between the two surfaces, and therefore resulting in a backward curvature.

TABLE 1
GROWTH OF PEA SECTIONS WITH AND WITHOUT EPIDERMIS
AT DIFFERENT EXTERNAL PHs

SECTIONS	TIME OF MEASUREMENT (HOURS)	GROWTH IN LENGTH IN MM.*	
		pH 7.0	pH 4.0
With epidermis.	$\begin{cases} 2 \\ 20 \end{cases}$	$\begin{matrix} 0.50 \\ 0.78 \end{matrix}$	$\begin{matrix} 0.53 \\ 0.90 \end{matrix}$
Without epidermis.	$\begin{cases} 2 \\ 20 \end{cases}$	$\begin{matrix} 0.68 \\ 0.60 \end{matrix}$	$\begin{matrix} 1.07 \\ 1.06 \end{matrix}$

* Values are averages of ten sections.

TABLE 2
RELATION OF INTERNAL TO EXTERNAL pH OF PEA SECTIONS
WITH AND WITHOUT EPIDERMIS

TYPE OF SECTION	NO. OF SECTIONS	EXTERNAL pH	INTERNAL pH
Fresh intact plants.	30	6.25
Split part of sections in buffer solution eighteen hours ...	30	$\begin{matrix} 7.0 \\ 6.0 \\ 5.0 \\ 4.0 \end{matrix}$	$\begin{matrix} 6.41 \\ 6.18 \\ 6.08 \\ 5.70 \end{matrix}$
Intact sections in buffer solution eighteen hours.	30	$\begin{matrix} 8.0 \\ 4.0 \end{matrix}$	$\begin{matrix} 6.10 \\ 6.17 \end{matrix}$

Evidently the acid curvature is caused by a higher active auxin concentration on the cut surface than on the surface with the intact epidermis.

RELATIONSHIP BETWEEN pK OF A GROWTH SUBSTANCE AND ITS ACTIVITY

The three substances used for the main body of this work are indole(3)acetic acid (pK = 4.75), phenylacetic acid (pK = 4.25),

and *cis*-cinnamic acid ($pK = 3.85$). The structures of these substances are shown in figure 2. The curves obtained for each of these substances by plotting degrees curvature in the pea test against the pH of the external solution are shown in figure 3, and the actual values for the curvatures are presented in table 3. These values

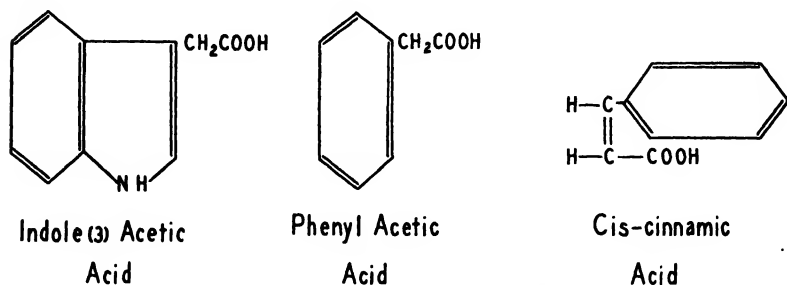


FIG. 2.—Three auxins active in the pea test

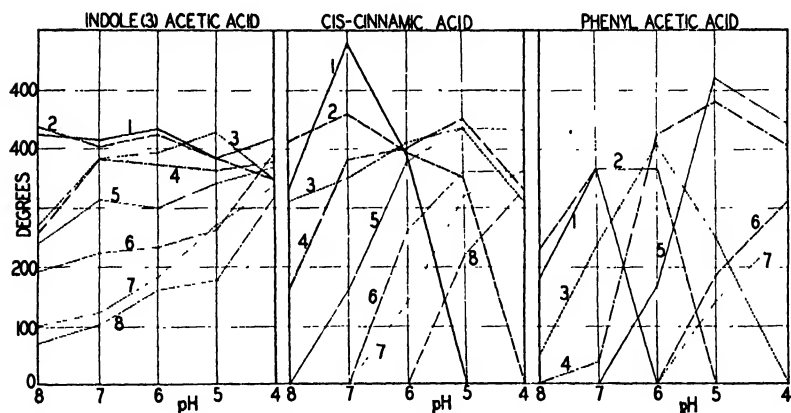


FIG. 3.—Effect of external pH on activity of auxins in the pea test. Curve numbers correspond to those in table 3.

represent the observed curvatures corrected for acid curvature. Obviously this must be done since at the more acid pH the observed curvature represents the resultant of two antagonistic reactions. At pH 7.0 there is only growth curvature, so that as the pH decreases these two reactions will tend to counteract each other, giving an apparent curvature much smaller than the true inward curvature.

Three points of interest can be noted in the curves presented in figure 3. (1) The slope of the curves obtained for phenylacetic acid and cis-cinnamic acid are much steeper than that for indole(3)acetic

TABLE 3

EFFECT OF EXTERNAL pH ON ACTIVITY OF AUXINS IN THE PEA TEST

CURVE NO.	MOLAR CONCENTRATION	PEA CURVATURE*				
		pH 8.0	pH 7.0	pH 6.0	pH 5.0†	pH 4.0†
1	Indole (3) acetic acid					
	2.0×10^{-4}	420	410	430	380	420
2	1.0×10^{-4}	430	400	420	380	350
3	5.0×10^{-5}	270	380	390	380	350
4	2.5×10^{-5}	260	380	360	360	380
5	1.2×10^{-5}	240	310	300	340	370
6	6.0×10^{-6}	190	220	230	260	390
7	3.0×10^{-6}	100	120	180	270	340
8	1.5×10^{-6}	70	100	160	150	320
1	Phenylacetic acid					
	32.0×10^{-4}	180	360	100	Toxic	Toxic
2	16.0×10^{-4}	230	360	370	0	Toxic
3	8.0×10^{-4}	50	240	410	250	0
4	4.0×10^{-4}	0	30	420	480	410
5	2.0×10^{-4}	0	0	170	450	450
6	1.0×10^{-4}	0	0	0	180	310
7	5.0×10^{-5}	0	0	0	140	260
8	2.5×10^{-5}	0	0	0	0	0
1	Cis-cinnamic acid					
	16.0×10^{-4}	330	580	380	Toxic	Toxic
2	8.0×10^{-4}	410	460	390	350	Toxic
3	4.0×10^{-4}	310	350	410	430	310
4	2.0×10^{-4}	160	380	360	450	330
5	1.0×10^{-4}	0	160	320	430	430
6	5.0×10^{-5}	0	0	260	360	360
7	2.5×10^{-5}	0	0	140	320	360
8	1.2×10^{-5}	0	0	0	220	330

* Each value averages six sections, or twelve separate curvatures.

† Observed values of curvature have 100° correction added for pH 5.0, and 210° for pH 4.0.

acid. (2) In the higher concentrations of phenylacetic acid and of cis-cinnamic acid extreme inhibition and toxicity occur at the lower pHs, while with indole(3)acetic acid slight inhibition occurs only at the highest concentration. (3) At a pH of 5.0 a concentration of

10^{-4} molal is just inhibitory for indole(3)acetic acid and for cis-cinnamic acid, while for phenylacetic acid a concentration of 2×10^{-4} molal is just inhibitory at pH 5.0. Each of these three points will be discussed later in the paper in the light of further considerations.

It was deemed advisable to determine whether a correlation exists between the pK of a growth substance and its activity in the pea test. This general type of correlation has been shown by J. BONNER (1) only in the *Avena*, and only for the natural auxin of the plant. Tests were made upon the two substances phenylacetic acid and cis-cinnamic acid, in concentrations that would give approximately the same reaction in the pea test, and from an external pH of 7.0 to 3.8 in intervals of 0.4 of a pH unit. The internal pH resulting from each external pH was determined and was used for correlative purposes. The pea curvatures were plotted against pH, and the dissociation curve was fitted to this. In figure 4 it may be seen that the correlation between the pK of a growth substance and its activity in the pea test is very good up to concentrations where inhibition sets in, for phenylacetic acid and for cis-cinnamic acid. This might well serve as an indication that these two substances take part in a manner similar to auxin in some pH dependent reaction.

As was shown by WENT (10), the curvature in the pea test is an exponential function of the concentration of the growth substance. By plotting the logarithm of the concentration against pea curvature, one obtains a straight line over a rather wide range of concentrations. For each pH, the logarithm concentration was plotted against curvature for the data presented in figure 3. It was seen that the curves obtained for each substance were similar, but that the curve for cis-cinnamic acid was shifted to the right (toward higher concentrations) of that obtained for indole(3)acetic acid, and the curve for phenylacetic acid was shifted even more so. This difference in activity between indole(3)acetic acid and cis-cinnamic acid was maximal at pH 8.0 and minimal at pH 4.0, showing that the pH effect is much more marked for cis-cinnamic acid than for the other acid. As mentioned earlier, J. BONNER, from work on the effect of acid on growth in the *Avena* coleoptile, postulated that auxin was active principally in the non-dissociated form. In order

that a substance with a pK less than that of auxin elicit the same growth response, a higher total concentration of this acid must be present in the cell in order that an equivalent amount of non-dissociated molecules be present. A correction for this difference in

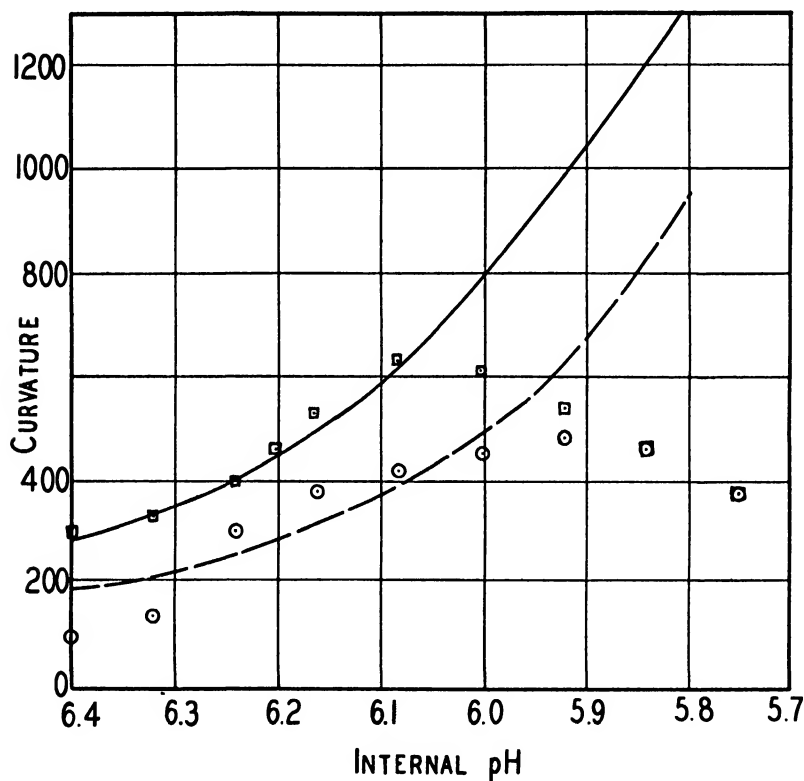


FIG. 4. —Correlation of internal pH, activity, and of pK :

— dissociation curve of cis-cinnamic acid.

- - - dissociation curve of phenylacetic acid.

□ pea curvature of cis-cinnamic acid.

○ pea curvature of phenylacetic acid.

degree of dissociation must thus be made before the activity of two substances with different dissociation constants can be compared. This can readily be done, taking for example two hypothetical substances *A* and *B*, *A* possessing the higher activity and the higher pK . To correct them to the same degree of dissociation at a given

pH, the observed activity of *B* must be multiplied by the ratio of the degree of dissociation of *A* to that of *B*, at the given pH. The degree of dissociation can be readily computed if the pK of the substances and the pH of the solution are known. This correction was made on the data presented in table 3, and the result is shown in figure 5. Figure 5 was computed at a pH of 6.2 (pH of the cell sap)

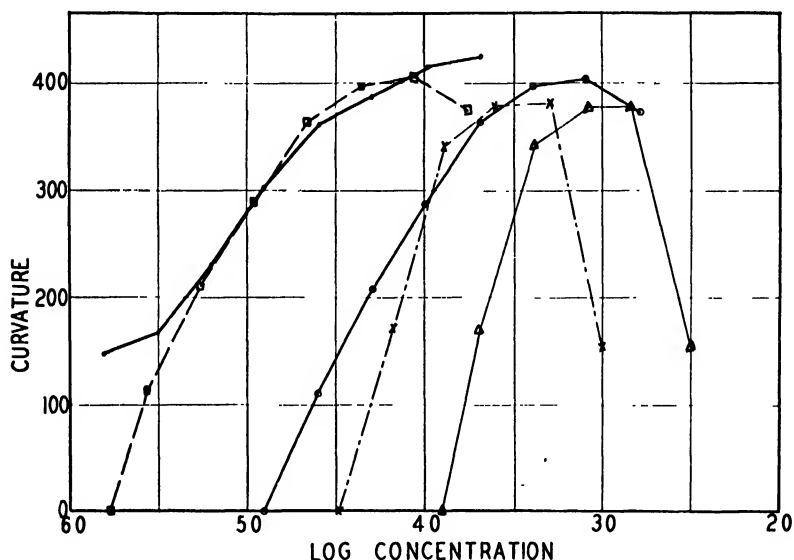


FIG. 5.—Activities of auxins corrected to the same percentage dissociation as that of indole(3)acetic acid at pH 6.2:

--- indole(3)acetic acid.

○—○ cis-cinnamic acid.

△—△ phenylacetic acid.

□---□ cis-cinnamic acid corrected to the same percentage dissociation as indole(3)acetic acid.

×---× phenylacetic acid corrected to the same percentage dissociation as indole(3)acetic acid.

by interpolation between pH 7.0 and 6.0. The experiment was repeated several times at pH 6.2, and the same result was always observed, as shown by figure 6. In figure 5 it is seen that over the major portion of the curve cis-cinnamic acid, when corrected for difference in degree of dissociation, has exactly the same activity as indole(3)acetic acid, differing only in falling off to zero activity at

higher concentrations than does indole(3)acetic acid and showing toxicity at the higher concentrations of free acid. Although the activity of phenylacetic acid was greatly enhanced by application of

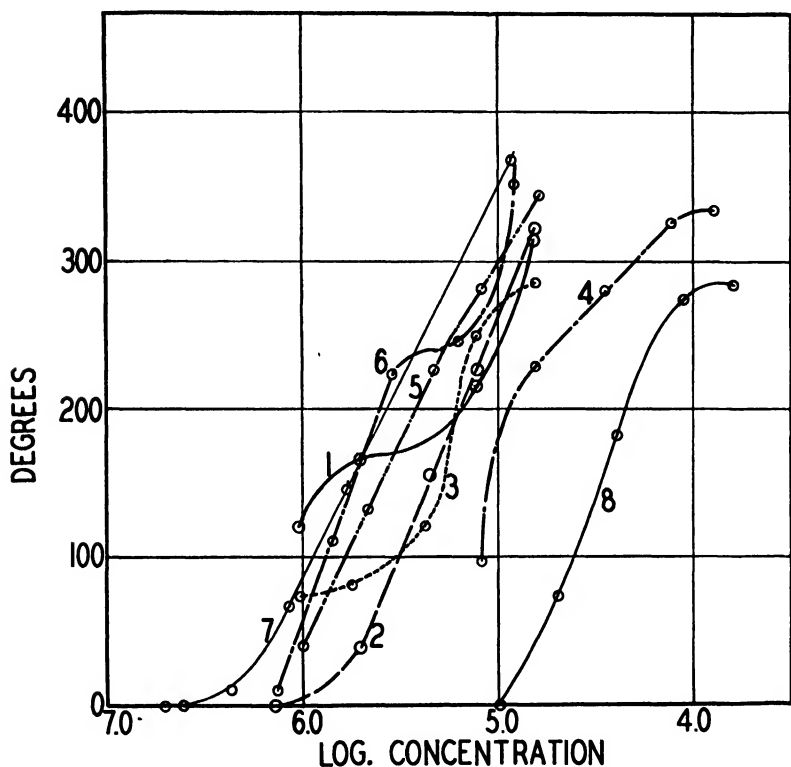


FIG. 6.—Activities of auxins corrected for differences in activities in pretreatment reaction, and for differences in dissociation constants.

1, indole(3)acetic acid.

2, indole(3)propionic acid.

3, indole(3)butyric acid.

4, indole(3)valeric acid.

5, naphthaleneacetic acid.

6, anthraceneacetic acid.

7, cis-cinnamic acid.

8, phenylacetic acid.

pH, 6.2.

this correction, it was not brought to an activity equivalent to that of indole(3)acetic acid.

The explanation of the toxicity of cis-cinnamic acid is probably the following. In order that cis-cinnamic acid and indole(3)acetic

acid have present in the cell equal amounts of free acid required to elicit a given response, the total concentration of *cis*-cinnamic acid must be greater than that of indole(3)acetic acid since *cis*-cinnamic acid possesses the smaller *pK*. As mentioned earlier, both acids become toxic at the same total external concentration 10^{-4} molal, which might indicate that they enter the cell to the same extent. With the higher concentration of *cis*-cinnamic acid necessary to give the same growth response, the concentration of hydrogen ions in the cell must be greater with *cis*-cinnamic acid than with indole(3)acetic acid, thus changing the internal *pH* sufficiently to cause injury to the cells.

The observation that *cis*-cinnamic acid has the same molar activity as indole(3)acetic acid, if the physiological processes in which it must take part are limited in number (as in the pea test), and if corrections are made for differences in physical properties, leads to the view that under proper circumstances many substances might well possess equal activities upon cell elongation. WENT during the progress of this work made the interesting observation that two independent reactions are concerned in the pea test, (1) the growth reaction, and (2) a reaction strongly influenced by phenylbutyric acid, a substance showing no growth response whatever. This was demonstrated by pretreating pea sections with phenylbutyric acid, thereby greatly increasing their sensitivity to auxins. It seemed logical then that of the numerous compounds tested in the pea test many were not limited by reactivity in the growth reaction, but by their reactivity in the pretreatment reaction. Several compounds were tested, therefore, using sections pretreated for two hours with phenylbutyric acid at a concentration of 100 mg./liter, and buffered at *pH* 6.2. Buffering was used merely to prevent excessive acid curvatures. Using this method, and correcting for differences in *pK* when possible, the following compounds were found to possess the same activity at *pH* 6.2 as indole(3)acetic acid (fig. 6). Indole(3)propionic acid, indole(3)butyric acid, naphthaleneacetic acid, anthraceneacetic acid, and *cis*-cinnamic acid. The activities of indole(3)valeric acid and of phenylacetic acid, although enhanced over previously reported values (3, 6), were not brought to the activity of indole(3)acetic acid. By using this pretreatment with phe-

nylbutyric acid, cis-cinnamic acid no longer dropped to zero activity before indole(3)acetic acid, showing that at lower concentrations the activity of cis-cinnamic acid in accessory processes had become the limiting factor. It is now possible to advance a hypothesis for the difference in the slopes of the curves shown in figure 3, where degrees curvature in the pea test were plotted against pH for the three different growth substances. There is definitely more than one process concerned in the pea test, and these probably are not all affected by pH. In fact it has been shown that the process influenced by pretreatment with phenylbutyric acid is independent of pH. For substances having equal activities in a pH dependent reaction but different activities in a pH independent, their behavior with change of pH will be a function of their relative activities in each of the two processes. The substance with the least overall sensitivity to pH will then have the smallest slope. The activity of indole(3)acetic acid is not accurately determined in the pretreatment reaction, so at present little can be said as to the relative activities of indole(3)acetic acid, phenylacetic acid, and cis-cinnamic acid in this reaction. In case it actually possesses a low activity in the pretreatment reaction, this would give an explanation for the difference in slopes noted in figure 3.

Discussion

The properties that a growth hormone of the auxin group must possess might be broadly classified into two general groups: ability to take part in a stoichiometric reaction which results in cell elongation, and ability to furnish the necessary "environment" for this reaction (1). For example, it must be able to take part in the "pretreatment reaction." In addition to these two properties there is the third property of transportability discussed by THIMANN (8). Any one of these three factors may, depending upon the method of assay, limit the apparent activity of a substance, and thereby obscure its activity in the primary reaction (1) already mentioned. Thus THIMANN showed that the activity of a substance was limited in the *Avena* test by its transportability, so that by the use of the pea test where this plays no part many more compounds were shown to possess growth activity, although to varying extents. For example,

cis-cinnamic acid was found to have 10 per cent (7)² of the activity of indole(3)acetic acid. It has been shown in the present paper that if corrections for the differences in pKs are made so that equimolar concentrations of free acid are compared, cis-cinnamic acid has an activity per molecule equal to that of indole(3)acetic acid. Still another reaction may limit the activity of a substance in the pea test. This reaction is a preparatory reaction, and as mentioned above can be carried out by substances having no true growth activity; for example, by phenylbutyric acid. If this reaction is made non-limiting by pretreatment with phenylbutyric acid a still closer approach may be made toward measuring the primary growth activity of a substance. In this way several more compounds were shown to possess the same activity per molecule as indole(3)acetic acid. It may be concluded from this that the difference in activities observed for different auxins in the pea test (3, 7) is due to their activities in various accessory reactions, but that in the final growth reaction, pH dependent and stoichiometric in nature, they all possess the same activity.

From the work of HAAGEN SMIT and WENT (3), KOEPFLI, THIMANN, and WENT (7), and of KÖGL and KOSTERMANS (6), an essential molecular structure for a growth promoting substance was formulated. The requirements are (1) a carboxyl group, (2) a ring structure, (3) one double bond in the ring structure, (4) a minimum distance of at least one carbon atom between the carboxyl group and the ring, and (5) a definite steric configuration. Inasmuch as no compounds lacking in these requirements have been found to give the growth reaction, it would seem that these are also the requirements for the final reaction. Thus one might conclude on the basis of the experiments reported here that all compounds possessing these basic structural requirements will be equally active in the reaction resulting in cell elongation, and any difference in activity will be due to varying activities in accessory reactions.

² HAAGEN SMIT and WENT (3) reported cis-cinnamic acid as having the same activity as indole(3)acetic acid in the pea test. They compared at pH 7.0 concentrations that gave high pea curvatures, and so were working at high concentrations where the pK factor is less important as may be seen from figure 5.

Summary

1. It is concluded that the acid curvature in the pea test is due to a greater growth of the cut surface than of the intact surface. This is explained as due to a higher concentration of active auxin on the cut surface, caused by a measurable decrease of the internal pH of the cut surface, when split pea sections are placed in acid buffers.

2. A correlation is found between the dissociation curves and the activities at different internal pHs of *cis*-cinnamic acid and phenylacetic acid.

3. By correcting for difference in pK, so that only equimolar concentrations of the free acid are compared, it is found that *cis*-cinnamic acid possesses the same activity as indole(3)acetic acid in the pea test. The activity of phenylacetic acid, although enhanced, was not so high as that of indole(3)acetic acid.

4. By pretreating pea sections with phenylbutyric acid and correcting for difference in pK when possible, the following compounds are found to possess molar activities equal to that of indole(3)acetic acid in the pea test: indole(3)propionic acid, indole(3)butyric acid, naphthaleneacetic acid, anthraceneacetic acid, and *cis*-cinnamic acid. The activities of phenylacetic acid and of indole(3)valeric acid, although increased, are not brought to that of indole(3)acetic acid.

5. It is suggested that compounds possessing the essential molecular structure probably all have the same activity in the pH dependent, and stoichiometric growth reaction, and that the differences in their observed activities are due to differences in activities in secondary processes.

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ORIGIN AND DEVELOPMENT OF SHOOTS FROM THE TIPS OF ROOTS OF *POGONIA* *OPHIOGLOSSOIDES*

MARGERY C. CARLSON

(WITH THIRTEEN FIGURES)

Introduction

While observing plants of *Pogonia ophioglossoides* (L.) Ker. in a sphagnum bog in northern Wisconsin, it was noticed that the new shoots arose from, or near, the tips of the roots. Adventitious buds are of common occurrence on roots, many plants being propagated by root cuttings; but only a few cases of shoot formation similar to that in *Pogonia* have been recorded.

BEIJERINCK (1) distinguished between those adventitious buds which originate from the phellogen, or from the wound callus, of root cuttings and those formed normally, or after injury, from the primary tissues of the root, but studied only the latter. He described briefly the formation of buds from the outer layers of the cortex of the roots in *Aristolochia clematitis*, *Linaria vulgaris*, and *Orobanché galii* and other parasites. TRÉCUL (10) reported the endogenous production of shoots from root cuttings of *Maclura aurantiaca*, *Tecoma radicans*, *Ailanthus glandulosa*, and *Paulownia imperialis*. WARMING (13) found buds arising in the outer cortical layers in certain members of the Podostemaceae. PRIESTLEY and SWINGLE (7) described in detail the origin and development of adventitious buds in root cuttings of *Crambe maritima*, in which certain cells of the phellogen near a wound become more actively meristematic than their neighbors and produce a group of dividing cells which soon differentiate into the growing point of a bud. GRAHAM and STEWART (3) described stages in the development of buds from the cambium of root cuttings of *Anchusa italica*.

The few cases where the tip of the root becomes transformed into a shoot have been summed up and described by ROSTOWZEW (9). In *Neottia nidus-avis*, PRILLIEUX (8), WARMING (12), IRMISCH (5),

and others found that a swelling appears, either on the end of the root or laterally just back from the tip, and differentiates into a bud with alternating, semicircular leaf primordia and with a cluster of roots arising from the base of the bud as it elongates into a shoot. If the bud is lateral, the tip of the main root continues to grow forward. It was suggested that these adventitious "root buds," with their roots, may provide a method of vegetative propagation for this plant; but BEIJERINCK and IRMISCH state that they are only occasionally produced in nature, and that when produced they often die prematurely. BEIJERINCK noted that adventitious roots were produced exogenously in great numbers from the rhizomes of *Neottia*, and suggested that there might be a close anatomical relationship between the stem and the root in this plant, as in *Corallorhiza* and *Epipogium*. GOEBEL (2) found on three plants of *Anthurium longifolium* that terminal "root sprouts" were produced in a manner very similar to that in *Neottia*. KARSTEN (6) reported the production of flowers from a swelling on the end of the root of a plant of *Dioscorea* in a greenhouse in the hot summer of 1858. SACHS claimed that the tip of the root of *Platynerium willinkii* becomes transformed into a bud, and ROSTOWZEW (9) described fully this transformation in the preceding and in other species of *Platynerium* and in *Asplenium esculentum*. VAN TIEGHEM (11) and HOLLE (4) described the formation of a shoot from the tip of the root in *Ophioglossum vulgatum*. They reported that a root develops endogenously from the adventitious shoot and grows in the direction of the mother root, thereby making the shoot appear to arise laterally from the main root, as a sort of "embryo" formed from the root meristem.

A peculiar situation appears in *Rumex acetosella*, which according to BEIJERINCK (1) ordinarily produces buds endogenously in a ring about the base of a secondary root, when it grows horizontally close to the surface of the ground; but which on one occasion produced a bud on a root cutting, the bud then becoming transformed into a root. His figures show a branch root with root cap and root hairs, having a ring of reduced leaves at its base, and he suggested that the bud began to develop, then suddenly changed its growing point into that of a root tip. Other investigators express doubt as to the accuracy of these observations.

Investigation

This study was made from hand sections of fresh material and from stained serial sections of material imbedded in paraffin.

The roots of *P. ophioglossoides* grow horizontally in the sphagnum mat just above the level of the standing water in the bog. They are 1 to 1.5 mm. in diameter throughout their length, are unbranched,

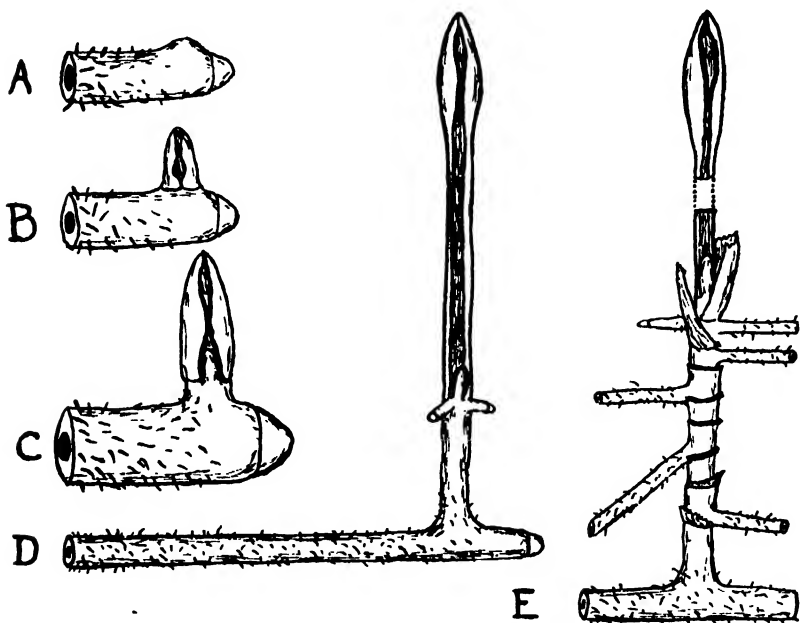
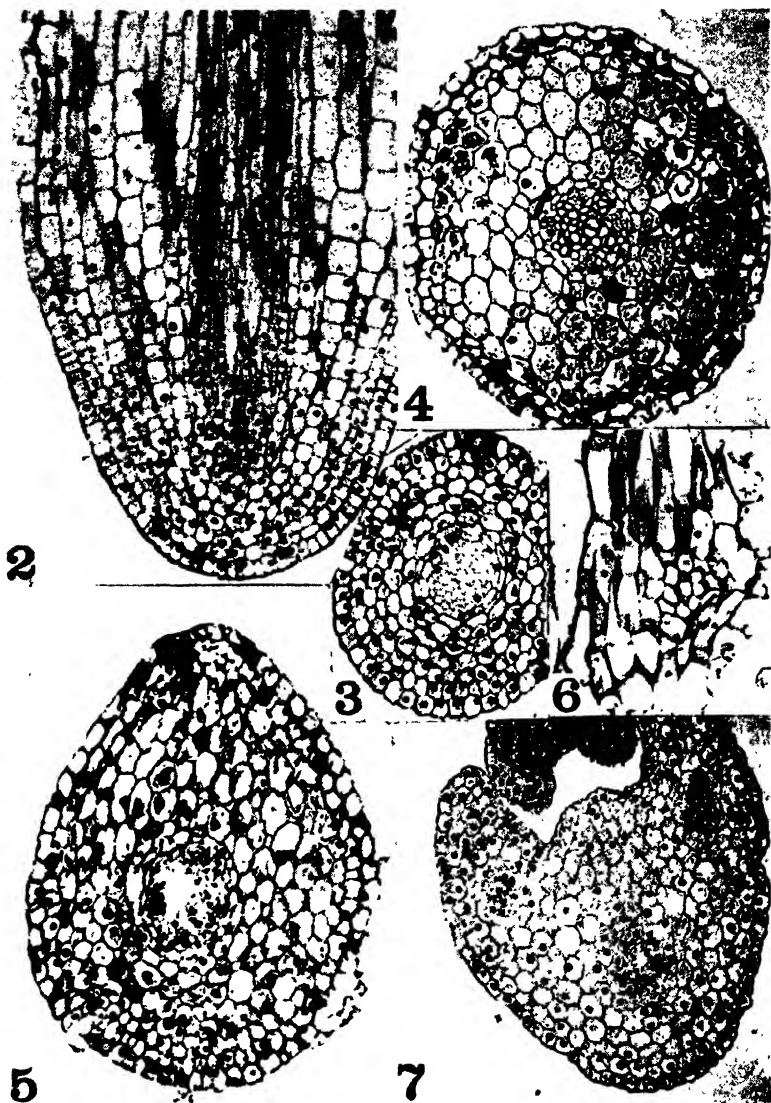


FIG. 1.—A: portion of end of root showing protuberance on upper side. B: same, protuberance differentiated into young shoot. C: older shoot showing elongated outer leaf, primordium of second leaf, and slight elongation of axis below first node. D: still older shoot showing differentiation of outer leaf into petiole and blade, second leaf further developed, and two adventitious roots arising from first node. E: much older shoot showing nine nodes, each with leaf or leaf scar and sometimes with one or two adventitious roots; tip of main root grown forward; root hairs persisting.

coarsely hairy, and are usually green. The short tapering tips are smooth. A longitudinal section of the tip of the root (fig. 2) shows its unusual structure. No root cap is present. The promeristem is shorter and the cells are more vacuolate, their cytoplasm less dense, and their nuclei smaller in proportion to the size of the cells than is typical for such regions in roots. Differentiation of the histogens be-



FIGS. 2-7.—Fig. 2, longitudinal section of tip of root showing early differentiation of tissues; no root cap present. Fig. 3, cross section of root through region of primary meristems showing beginnings of tissue differentiation. Fig. 4, same through mature region showing tetrarch xylem and phloem; mycorrhizal fungus in some and starch grains in other cortical parenchyma cells; thick walls between epidermis and hypodermis. Fig. 5, cross section of root showing cell enlargement on one side; at peak of swelling the cells have divided; an early stage in shoot formation. Fig. 6, tangential section of root showing group of dividing cells in cross section. Fig. 7, cross section showing two leaf primordia of developing bud, outer one showing three provascular strands; inner one showing no differentiation of tissues.

gins early. A well defined layer of epidermal cells, with their outer walls conspicuously thickened, completely covers the tip of the root, and the provascular strand becomes evident about 0.1 mm. back from the tip. Lateral enlargement and elongation of cells can be followed in figure 2. Distally from the place where lateral enlargement of cells reaches its maximum, the outer walls of the epidermal cells are thinner than below, and are frequently broken on the more mature parts of the root. The inner walls of these cells, however, are distinctly thicker here than in the meristematic region.

A cross section of the root at a level where the primary tissues are beginning to differentiate is shown in figure 3. Its protostelic nature is evident. The matured region (fig. 4) shows the radial and tetrarch arrangement of the primary xylem and phloem. The protoxylem consists of spiral and scalariform tracheids; the metaxylem consists of vessels, tracheids, and a few parenchymatous cells. The phloem, consisting of sieve tubes and parenchymatous cells, lies between the radiating strands of xylem. All the cells of the provascular strand mature into xylem and phloem, so that no cambium remains. The steles vary from triarch to hexarch, the tetrarch condition being most common. The pericycle and endodermis are single layered parenchymatous tissues. The Casparian strips in the endodermal cells are conspicuous. The cortex consists of from five to seven layers of loosely arranged parenchymatous cells, usually filled with starch grains. The outermost cortical layer (hypodermis) is more like the epidermis than the rest of the cortex: the cells are tightly fitted together and contain no starch grains. The walls between the hypodermis and the epidermis are much thicker than the outer walls of the epidermis.

Root hairs are produced in the usual manner from a few of the epidermal cells. Their walls are somewhat thickened, however, and they persist all along the root as a sparse, coarse fuzz.

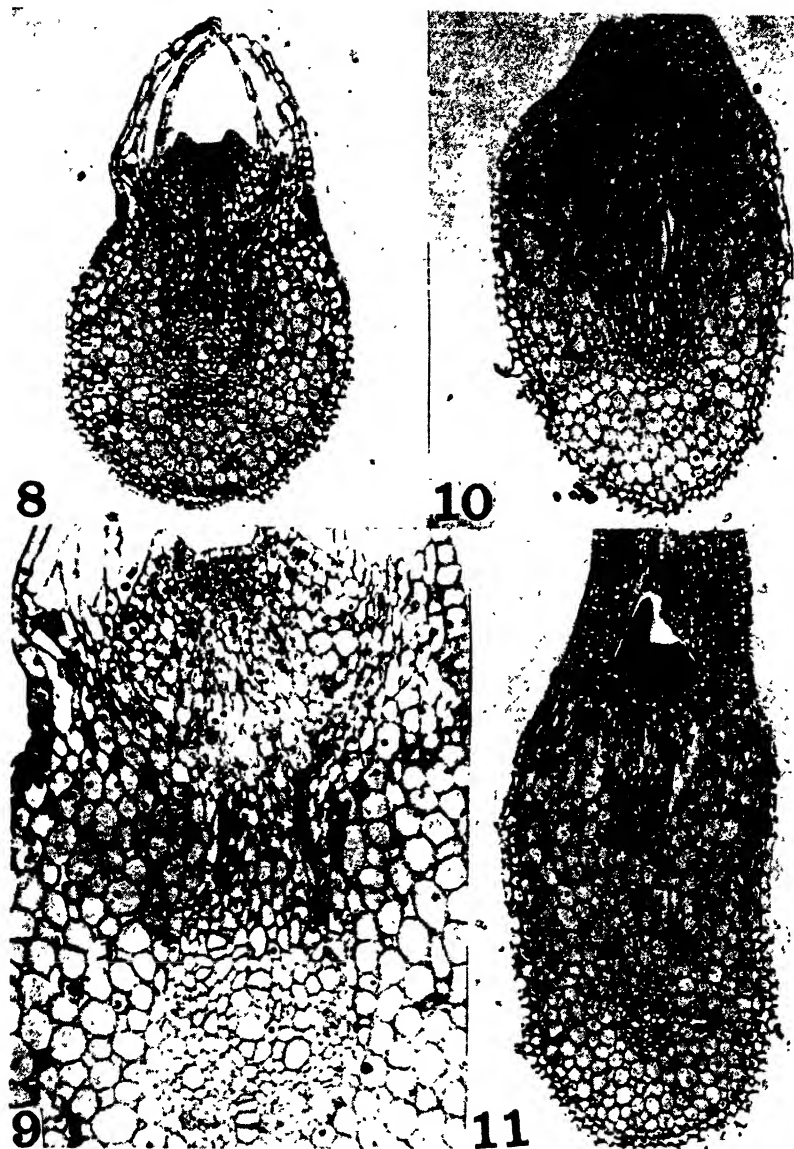
A mycorrhizal fungus enters the root hairs or external walls of other epidermal cells, passes through the hypodermis and often through one or two adjacent layers of cells, and forms a tangled mass of hyphae in the cells in the next several layers of the cortex (fig. 4). Upon entrance of the fungus into a cell, the reserve starch begins to disappear, the nucleus at first enlarges, and then becomes

indistinguishable in the mat of fungal hyphae. The mycelium seems never to penetrate deeper than the inner cortical cells.

The adventitious shoot begins its development as a protuberance on the side or on the end of a root (fig. 1*A*). The epidermal and cortical cells enlarge, chiefly in the radial dimension and form a swelling, at the peak of which the cells begin to divide. Since the daughter cells do not immediately enlarge, a group of small cells, destined to become a bud, is formed (figs. 5, 6). The protuberance enlarges as the cells continue to divide and form the promeristem of a stem tip (figs. 1*B*, *C*; 8), the outer part of which differentiates into a dome-shaped leaf (fig. 11). A second leaf begins as a semicircular ridgelike primordium arising from the promeristem (figs. 7, 9).

Elongation of the shoot begins with the radial and tangential enlargement of the epidermal and cortical cells of the root between the stele and the stem tip, early stages of which are seen in figures 1*C*' and 8, and successively later stages in figures 10 and 11. The cells of the lower side of the root have not changed at all. The outer sheathing leaf elongates (figs. 8, 11), the inner leaf primordium enlarges, and later leaf primordia form successively within one another.

A study of a series of cross sections of the shoot from the tip downward shows the differentiation of tissues and the transition between the tissues of the shoot and root. Three or more small provascular strands appear just below the promeristem in a ring surrounding a small pith region. At a lower level, but before much maturation of cells has occurred, three strands from the outer leaf extend through the cortex and join the ring of bundles. Farther down, where the cells of the stem are mature (fig. 12), there is an epidermis, the outer walls of whose cells are somewhat thickened; the cortical parenchyma, with starch grains and mycorrhizal fungus; the endodermis, with Casparian strips; the parenchymatous pericycle; the ring of three vascular bundles; three leaf traces in the cortex; and the small three pointed pith. The xylem of a bundle partially incloses a small strand of phloem, which lies toward the outside. The xylem of adjacent bundles is in contact. Neither the xylem nor the phloem is highly specialized. No cambium is present. The cells of the pith may become thick walled. The vascular tissues continue as described down to the stele of the root, where they



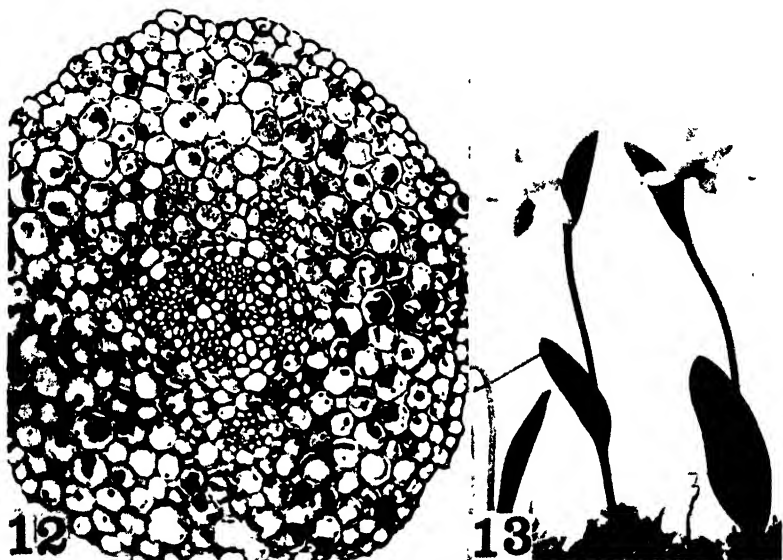
FIGS. 8-11.—Fig. 8, bud showing first two leaf primordia in longitudinal section on side of root; vascular tissues between bud and stele of root beginning to differentiate. Fig. 9, portion of bud shown in fig. 8 enlarged. Fig. 10, bud (not median section) showing early elongation by enlargement of cells between stele and bud; further differentiation of vascular tissues between root and bud; undifferentiated median trace of first leaf merging with transition vascular tissues. Fig. 11, later stage of growing shoot; first leaf elongating, especially at base; primordium of second leaf further developed than in fig. 8; greater elongation in region between root and first node.

merge with those of the root. Here the stele of the root is enlarged on the upper side by the production of much xylem (figs. 8, 9). The characteristic arrangement of xylem and phloem is evident, but the upper radial plate of xylem is much larger than the others, and the two strands of phloem on either side of it are displaced laterally by the added xylem tissue. The endodermis and pericycle are not differentiated about the stele on the side toward the developing bud, but the cells between the vascular tissues of the shoot and root mature so that a connection is established. It must be remembered that the differentiation of tissues occurs simultaneously in shoot and root; hence the connection of their xylem, phloem, pericycle, and endodermis is present from the beginning of differentiation. The pith of the shoot ends at the stele of the root. Epidermal hairs similar to those on the root are often present on the basal part of the stem.

Further growth and differentiation of root and shoot proceed normally. The part of the shoot below the first leaf may elongate so that the first node is 0.1 to 4.0 cm. above the root. The sheathing petiole of the first leaf elongates, pushing above the surface of the surrounding sphagnum. The upper end broadens and unfolds into a narrowly oval blade which is usually about 3-6 cm. long and 0.5-1.0 cm. wide when mature. The stem tip develops very slowly, and the second leaf, which lies in a groove of the petiole of the first leaf, gradually elongates, differentiates, and is pushed upward by elongation of the first internode.

While the first leaf is developing, one or more adventitious roots are usually produced at the first node (fig. 1*D*), just above or below the insertion of the leaf. The early stages in their development are very similar to those in the development of the adventitious buds. They are exogenous; that is, they originate from the epidermal and cortical cells of the stem, which at a certain point near the leaf insertion begin to divide, forming a group of meristematic cells which appears as a swelling on the surface. This group of cells becomes organized into a root tip which grows and extends outward at right angles to the stem. If the root is formed above the attachment of the leaf, it pushes through the petiole. Vascular tissues are differentiated in the developing root and in the cortex of the stem

connecting the new root with the stele of the stem. These adventitious roots grow horizontally, in a plane somewhat above that of the first root, and are similar to it in appearance, structure, and behavior. One leaf is produced at each node (fig. 1E), the lower ones dying and leaving only shriveled bases or scars. One or two green and coarsely hairy adventitious roots may be produced on each



FIGS. 12, 13.—Fig. 12, cross section just below first node of stem showing epidermis, cortical parenchyma with starch grains and mycorrhizal funi, endodermis, pericycle, three vascular bundles, three leaf traces (from first leaf) in cortex, and small three angled pith region. Fig. 13, flowering shoots showing sessile leaves on aerial stem and bracts subtending the flowers.

node. The root, or roots, at the first (lowermost) node are usually the first to show evidence of shoot formation near their tips.

The seasonal history of the growth of the shoots has not been worked out carefully, but one leaf seems to appear above the sphagnum mat each season, each successive one taller and larger than the previous one, until finally the terminal bud produces an aerial flowering stem, 20 to 35 cm. high, with two or three leaves: a long stalked leaf near its base, one without a petiole midway up; and a leaflike bract subtending the flower (fig. 13). The number of

years elapsing between the first appearance of a bud and the production of a flowering stalk from this bud is not known.

Each new series of roots, growing horizontally from the nodes of the shoots, occupies a plane in the sphagnum mat a little higher than that of the previous series, and the new shoots originating from the ends of these roots are therefore also higher. The distance apart of these successive layers of roots may bear a relationship to the rate of growth of the sphagnum, but no measurements are available to verify this point.

Summary

1. The roots of *Pogonia ophioglossoides* are horizontal, unbranched, covered with persistent root hairs, and green, at least at their extremities. They are protostelic, radial, triarch to hexarch, with endodermis having conspicuous Casparian strips, and root hairs. They have no root cap.

2. An adventitious shoot begins as a swelling on the root, either at its end or on the upper side a short distance back from the tip. Lateral shoots on roots are more common than terminal ones. The swelling is formed by enlargement and division of the cells of the epidermis and cortex of the root, usually on the upper side, producing a group of meristematic cells which differentiates into a typical bud.

3. The new shoot produces one leaf at each node, and probably one leaf each season, until the shoot is old enough to flower.

4. If the adventitious shoot is lateral, the root may grow forward and produce another shoot the following season. One or two adventitious roots are produced exogenously at most nodes of the adventitious shoot.

5. The formation of exogenous adventitious shoots from tips of roots and of exogenous adventitious roots from shoots is the normal habit of growth in this species of *Pogonia*, and is apparently of comparatively rare occurrence among plants.

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VITAMIN B₁ AND THE GROWTH OF GREEN PLANTS¹

JAMES BONNER AND JESSE GREENE

(WITH TWO FIGURES)

Introduction

Vitamin B₁ is of importance as a growth factor for the roots of higher plants. Pea roots will grow in vitro in an otherwise optimal nutrient solution only if an adequate supply of vitamin B₁ is present (1, 3), and the same is true for excised roots of tomato (12). Vitamin B₁ should be regarded both as a "vitamin" for the growth of roots in vitro and certainly as a phytohormone in the green plant. Earlier work in animal nutrition has shown that vitamin B₁ is present in the leaves (2). It is formed there only in the presence of light (9). It would seem logical to suspect that the root receives its supply of vitamin B₁, either directly from green leaves, or indirectly from the seed, where vitamin B₁ is stored (2).

When plants are grown under optimal external environmental conditions such that no external factor can be said to limit growth, it is nevertheless found that the growth rate of the plant is limited. It is clear that under optimal external conditions, certain internal factors must determine the plant's growth. The ability to synthesize vitamin B₁ would be one such internal factor. A plant supplied with optimal light intensity, temperature, amounts of nutrients, etc., might still fail to supply to its roots an amount of vitamin B₁ sufficient to enable the root to fulfil its full potential growth. This would then be a hypothetical specific case of limitation by an internal factor.

The results recorded in the present paper, while of a preliminary nature, nevertheless indicate that because of its function as a root growth factor, vitamin B₁ may be of some importance in practical agriculture.

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Investigation

VITAMIN B₁ AS A PHYTOHORMONE

The following experiment was designed to show directly that the root is dependent upon the green leaf for its supply of vitamin B₁. Pea seeds were germinated upon filter paper in a physiological dark-room. When the epicotyl had attained a length of approximately 4 cm., the seedlings were divided into two lots of 100 each, and the cotyledons were removed from all the plants. One lot of 100 seedlings was then planted in sand in the greenhouse and supplied daily

TABLE 1
RELATION OF VITAMIN B₁ CONTENT OF LEAF AND
ROOT TIP OF PEA PLANTS WITH
COTYLEDONS REMOVED

PLANT ENVIRONMENT	PLANT PART	VITAMIN B ₁ CONTENT: MG. $\times 10^6$			
		DAYS AFTER REMOVAL OF COTYLEDONS			
		0	4	6	8
In dark	1 cm. root tip.	15	15	15	18
	Apical bud and two apical leaves.	28	32	32	32
In light	1 cm. root tip.	15	16	34	46
	Apical bud and two apical leaves.	28	36	46	100

with nutrient solution (10). The other lot was kept in the darkroom and supplied nutrient solution containing sucrose. At the time of removal of the cotyledons, and after four, six, and eight days, root tips 1 cm. long were removed and assayed for vitamin B₁ by the *Phycomyces* test (6, 14). At the same time the apical buds and two apical leaves were assayed in the same way. The results of one representative experiment are shown in table 1.

The tips of the primary root as well as the apical buds of the primary axis (at the time of removal of cotyledons) were found to contain a relatively large amount of vitamin B₁. In the plants which remained in the dark, the vitamin content of both root tip and bud

with the apical leaves remained approximately constant. In the series in the light the vitamin B₁ content of the bud and young apical leaves increased rapidly. Over the same period of time the amount present in the root tip also increased. The higher vitamin B₁ content of the roots in the light series is correlated with its synthesis in the apical portion of the plant, and indicates that it is supplied to the roots from the green leaves.

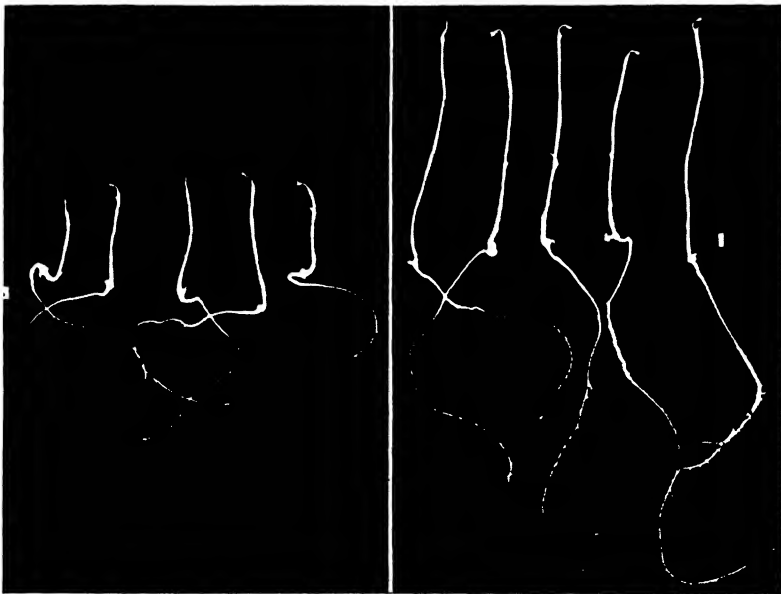


FIG. 1.—Pea embryos from which cotyledons were removed and then grown in dark under sterile conditions on nutrient medium of known composition. Left, without added vitamin B₁; right, with added vitamin B₁ (0.1 mg. per liter). Small rectangle indicates approximate size of initial embryo. Photographed after four weeks.

RELATION BETWEEN ROOT GROWTH AND SHOOT GROWTH

When excised pea embryos (without cotyledons) are grown *in vitro* in the dark upon a nutrient medium containing sucrose, nitrate, and other essential inorganic salts, the growth of the shoot is increased if small amounts of vitamin B₁ are added to the medium (4, 11), as shown in figure 1. Excised buds, however, grown *in vitro* upon the same medium, do not respond to added vitamin B₁ with increased growth. It seems probable, therefore, that whatever effect

vitamin B₁ exerts in increasing the growth of the shoot is due primarily to the effect of this substance upon root growth. WENT (18) has shown that shoot growth of etiolated pea seedlings from which the roots were removed is greatly decreased, even under conditions which assure an adequate food and water supply. According to WENT, some specific substance necessary for the growth of the shoot is formed in the active root, and when roots are cut off the locus of formation of this specific substance is therefore removed. There are of course other ways in which root activity might benefit shoot growth, in the case of the normal green plant.

EXPERIMENTS WITH GREEN PLANTS

Plants were grown in the greenhouse in washed sand in two gallon glazed crocks, provided with drainage. Two to several plants were grown in each crock, the number depending upon the type of plant. Selection carried out as carefully as possible assured that the control (without B₁) and the experimental (with B₁) plants were closely comparable. HOAGLAND'S (10) nutrient solution was used. This was supplied at the rate of 500 cc. per pot every two days and the pots flushed with tap water on the intervening days, frequent flushing being adopted in order to decrease as far as possible complications owing to the presence of microorganisms.

Vitamin B₁ was added to the nutrient solution in concentrations of 1, 0.1, and 0.01 mg. per liter.²

Pea (variety Perfection), bean (Kentucky Wonder), radish (Scarlet Globe), and tomato (Bonny Best) failed to show any significant response to vitamin B₁, when added to the nutrient solution in the concentration of 1 mg. per liter. It is realized that the cultural conditions of the present experiments were not ideal and the growth of these rapidly growing varieties may have been limited by nutrient supply rather than by internal factors. The fact that these plants grow rapidly and produce luxuriant root systems, however, indicates that they synthesize their internal growth factors (such as vitamin B₁) in amounts sufficient for ordinary growing conditions. In one experiment, beans grown in tank cultures with no limitation

² The writers are indebted to Merck & Co. for the supply of synthetic vitamin B₁ hydrochloride.

of mineral nutrients showed a considerable increase in rate of shoot growth in response to vitamin B₁ (0.1 mg. per liter).

Several varieties of plants noted as being slow growing were subjected to similar experimentation. The results are summarized in table 2. The tung oil tree (*Aleurites fordii*) is an example. Rooted cuttings (20) which had been potted for several months and whose growth had entirely ceased were used. The control plants grew well but the plants receiving vitamin B₁ (1 mg./liter of nutrient) showed

TABLE 2
EFFECT OF VITAMIN B₁ UPON SHOOT GROWTH OF PLANTS •
GROWN IN SAND CULTURE

SPECIES	NUMBER OF PLANTS	B ₁ CONCENTRATION (MG./L.)	DURATION OF EXPERIMENT (WEEKS)	AVERAGE SHOOT GROWTH, CM./PLANT FOR TOTAL PERIOD		
				CONTROL	VITAMIN B ₁	VITAMIN B ₁ CONTROL
<i>Aleurites fordii</i> *	6	1	10	7	13	1.86
<i>Buginvillea glabra</i> †	6	1	10	10.7	14 0	1 3
<i>Arbutus unedo</i> †	8	1	10	10.1	13.3	1.3
<i>Eucalyptus ficifolia</i> †	6	1	9	11.1	16 3	1 5
<i>Camellia japonica</i> ‡	24	0.1	5	0.0	2 0
<i>Camellia japonica</i> ‡	0.01	5	0.0	2 4

* Rooted cuttings.

† Selected seedlings kindly supplied by Dr. W. Lammerts, Armstrong Nurseries, Ontario, Calif.

‡ Seedling plants kindly supplied by Mr. J. Youtz.

almost twice as much total shoot elongation after two and one-half months. Upon removal of the plants from the crocks at this time, it was found that the root systems of the vitamin B₁ treated plants were more luxuriant than those of the control plants (fig. 2). The response to vitamin B₁ was similar to that shown by etiolated pea embryos; root growth was improved, and correlated with this was considerable increase of shoot growth.

With the other plants listed in table 2, the effects of added vitamin B₁ are in no case great but are nevertheless definite, despite the small numbers of plants used. *Hardenbergia comptoniana* actually grew considerably less when vitamin B₁ was supplied to it. In view of the results with lower concentrations, as detailed later, it is appar-



FIG. 2.—Tung oil trees grown in washed sand with nutrient solution. Plants on right received no vitamin B₁; plants on left received vitamin B₁ (1 mg. per liter). See table 3.

ent that a concentration of 1 mg. per liter is much higher than necessary, and might in some cases prove toxic.

Twenty-four selected *Camellia* plants approximately 17.5 cm. tall were divided into three lots and were then supplied respectively with no vitamin B₁ or with concentrations of it at 0.1 or 0.01 mg. per liter. All the plants were in the temporary resting state. During the five weeks of the experiment none of the control plants resumed growth, whereas seven of those treated with 0.01 mg./liter became active and grew in length an average of 2.4 cm. per plant.

TABLE 3
EFFECT OF VITAMIN B₁ ON SHOOT GROWTH OF PLANTS
GROWN IN SOIL

SPECIES	TOTAL PLANTS (WEEKS)	DURATION OF EXPERI- MENT	B ₁ CON- CENTRATION (MG./L.)	AVERAGE SHOOT GROWTH, CM./PLANT		
				CONTROL	VITAMIN B ₁	VITAMIN B ₁ CONTROL
<i>Arbutus unedo</i>	7	8	1	2.7	6.1	2.3
<i>Prunus ilicifolia</i>	11	8	1	4.0	6.4	1.6
<i>Bryophyllum</i> sp.	50	5	1	6.72	1.78	2.5

Other varieties showed increased shoot growth when vitamin B₁ was added to the soil every other day in the water given to the plant (table 3). *Arbutus unedo* and *Prunus ilicifolia* were grown outdoors in pots under conditions approximating ordinary nursery treatment. A comparison of tables 2 and 3 shows that *Arbutus* grows much more slowly under these conditions than in more favorable greenhouse sand culture environment, but nevertheless responds vigorously with increased shoot elongation when vitamin B₁ is added.

Bryophyllum daigremontiana and *B. tubiflora*, grown in the greenhouse in light potting soil, responded to added vitamin B₁ with considerable increases in rate of shoot and leaf development. Although the experiment recorded in table 3 was done with a 1 mg./liter solution of vitamin B₁, a second experiment with 0.01 mg./liter also showed a beneficial effect. The lowest limit of effective concentrations has not yet been determined.

ORGANIC FERTILIZERS AND OTHER SOURCES OF VITAMIN
B₁ IN THE SOIL

Although a wide variety of organic substances are to be found in soil (15, 16), yet their entire significance or importance has remained uncertain. In practice, organic fertilizers such as stable manure are often of distinctly more benefit to plant development than is a purely inorganic fertilizer. RUSSELL (13) states, "There is no doubt that the plant can grow satisfactorily and attain full development with inorganic nutrients only. But numerous field observations suggest that in the soil some of the organic matter may

TABLE 4

VITAMIN B₁ CONTENT OF MANURE AND OTHER MATERIALS

MATERIAL	VITAMIN B ₁ CONTENT*
Arizona steer manure†	0.13 mg./kilo
Local steer manure	0.08
Dairy manure	0.13
Alfalfa plant	5.0
Azotobacter‡	140

* All figures based upon air-dry weight of sample.

† Manure samples through courtesy of Dr. A. D. Shamel, U.S. Dept. Agr., Riverside, California.

‡ Sample of *Azotobacter* grown upon synthetic medium, through courtesy of Dr. G. Hilbert, U.S. Bureau of Chemistry and Soils, Washington, D.C.

play a direct part. No combination of artificial fertilizers is as effective as farm yard manure in maintaining a high uniform level of crop production from year to year." With no desire to oversimplify the problem of the effectiveness of such manures, the writers desire to point out one specific manner in which manure, aside from its content of the usual nutrients, may benefit the plant. As shown in table 4, manure of different varieties contains appreciable amounts of vitamin B₁, as determined by the *Phycomyces* test (6, 14).³

The samples tested contained approximately 5 per cent (or slightly more) of water soluble material and 0.1 mg. per kilo of vitamin B₁. On that basis the water soluble material from 1 kilo of manure dissolved in 10 liters of water would contain sufficient vitamin B₁ to exert marked effects upon plant growth (tables 2 and 3). As already mentioned, the lowest effective limit is not known, and it is

³ We are indebted to DAVID BONNER for carrying out the extraction and fractionation of the manure samples. Details of the work will be published in another connection.

possible that still more dilute solutions of vitamin B₁ may suffice for green plants. A nutrient solution containing 1 mg. of vitamin in 5000 liters exerts a maximum effect in supporting the growth of excised pea roots; one ten times more dilute still possesses a detectable influence (3). Purified extracts of manure (1 mg. of the absolute alcohol fraction of steer manure, representing 200 mg. of air-dry manure, per liter of nutrient medium) were also found to replace pure vitamin B₁ in supporting the growth of isolated pea roots in optimum nutrient solution.

Vitamin B₁ may reach the soil from still other sources. Plant residues contain considerable amounts, for example, and a portion may be returned to the soil after the death of the plant. Soil microorganisms are another possible source. *Azotobacter* cultured on purely synthetic medium is capable of synthesizing considerable amounts of vitamin B₁, and hence is to be regarded as "autotrophic" for this growth factor.

The vitamin B₁ economy of the soil can hardly be dealt with at this time, since it is undoubtedly a complex balance, increased on the one hand by organisms such as *Azotobacter* which produce vitamin B₁ and decreased on the other hand by the vitamin B₁ heterotrophic microorganisms and by some roots.

Discussion

That the growth of many green plants may be limited by the amount of available vitamin B₁ would be expected upon the basis of other experiments of a different nature. It has been shown that for the production of roots on cuttings, auxin, vitamin B₁, and other factors as well are necessary (17, 18, 19). Auxin serves only for the initiation of root primordia upon the cutting; for the development of these primordia into roots vitamin B₁ is essential, and root development may be strictly limited by the available amount of this substance. *Camellia* is one example of such a plant. Leafy cuttings of *Camellia* in the experience of this laboratory produce luxuriant roots only if vitamin B₁ is given after roots have been initiated by auxin treatment (17). *Camellia* is apparently unable to supply from its leaves sufficient vitamin B₁ for the abundant production of roots on cuttings, and the entire rooted plants respond with increased growth

to the application of vitamin B₁ (table 3). It would seem logical to conclude that *Camellia* normally has available only limited supplies of the vitamin. The leafy cuttings of plants capable of developing visible roots with auxin treatment alone (or even without such treatment) apparently already contain this vitamin, or are capable of manufacturing it in adequate amounts.

The fact that many plants benefit from an external supply of vitamin B₁ by no means indicates that this substance is not a plant hormone. In an analysis of the response of plants to vitamin C, it has been shown (5) that those varieties which respond favorably to additions of the substance are those whose capacity for synthesis of it is limited. Plants which synthesize large amounts of vitamin C gave no response to further additions. Vitamin C, however, is certainly to be regarded as a hormone for all plants. The different responses of different plants to vitamin B₁ have not yet been analyzed in a similar manner. It seems safe to predict, however, that many plants produce abundant supplies of this substance and are limited in growth by deficiency of it only under exceptional conditions. On the contrary, other plants almost as certainly produce smaller amounts of vitamin B₁ and their growth is limited by the available supply of it.

It is not desired to overemphasize the role of vitamin B₁ as an "organic fertilizer." The part which specific organic chemicals, special growth factors, present in fertilizers and in the soil may play in plant development has been much overlooked in the recent past. It is hoped that this specific example of such an organic "micronutrient" may serve to induce further investigation in this direction. Not only vitamin B₁, but a host of other specific growth substances—for example auxin, biotin, ascorbic acid, oestrogenic substances, etc. (2)—may benefit the growth of green plants under some conditions. The long disparaged "auximones" of BOTTOMLEY (7, 8) seem actually to rest upon a sounder basis than has been admitted in the past.

Summary

1. The vitamin B₁ content of pea plants kept in the dark does not increase, whereas in the light this content of the leaf rises rapidly. The root tips of plants whose leaves are in the light contain more

vitamin B₁ than do those of plants kept in the dark. This signifies that vitamin B₁ is produced in leaves in the light and thence transported to the growing root tip.

2. It has been shown that when vitamin B₁ is supplied in small amounts to the roots of plants grown in the dark, both shoot growth and root growth are increased.

3. Numerous species of plants have been grown in nutrient sand cultures in the greenhouse; that is, in the light. Addition of vitamin B₁ (1, 0.1, and 0.01 mg. per liter of nutrient solution) was found to increase the shoot growth of species of plants which are normally slow growing. No additional growth response to added vitamin B₁ was obtained under similar conditions when fast growing annual plants were used.

4. Organic manure contains appreciable amounts of vitamin B₁. The beneficial effects of manure upon plant development may be owing in part to its content of vitamin B₁. The vitamin B₁ content of soils may be expected to be derived also from plant debris and from soil microflora.

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PROTOPLASMIC STRUCTURE IN SPIROGYRA.
III. EFFECTS OF ANESTHETICS ON
PROTOPLASMIC ELASTICITY¹

HENRY T. NORTHEN

(WITH ONE FIGURE)

Anesthetics usually affect the rates of many biological processes. For example, at certain concentrations anesthetics cause an initial increase in the rate of respiration which may or may not be followed by a decrease, depending upon the concentration (2). HARVEY (1) suggests that the anesthetics may permit the mixing of certain enzymes and their substrates. He implies that the anesthetics may alter the structure of protoplasm.

NORTHEN (3) found that the rate with which the chloroplasts in cells of *Spirogyra* moved in response to different centrifugal accelerations followed the general equation:

$$V = k(c - c_0)$$

in which V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_0 the initial starting acceleration at which or below which the chloroplasts will not move regardless of the time the centrifugal acceleration is allowed to act. Because the rate of chloroplastic movement was governed by that equation, it was believed that the protoplasm in cells of *Spirogyra* was an elastic fluid built up of intermeshed linear molecules or micelles.

Stimuli which affect the rate of protoplasmic processes may conceivably alter the structure of protoplasm and the alterations so caused may be detected by changes in the value of c_0 , the initial centrifugal starting acceleration. NORTHEN and NORTHEN (4) have demonstrated that the value of c_0 is less than normal in groups of cells adjacent to mechanically killed or heat killed cells. The data

¹ Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 164.

in this paper indicate that anesthetics also cause an initial decrease in the value of c_0 .

Filaments of *Spirogyra* which had been immersed for different periods of time in several concentrations of various anesthetics were centrifuged, still in the proper anesthetic, with accelerations which were less than the average value of c_0 for untreated filaments. For each experiment about 200 filaments were used. The data were obtained by determining the percentages of filaments in the cells of which the chloroplasts had been displaced (chloroplasts moved to the end of the cell).

TABLE 1

EFFECT OF ETHYL BUTYRATE ON VALUE OF c_0 IN EQUATION $V=k(c-c_0)$

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF ETHYL BUTYRATE			
	0%	0.12%	0.25%	0.50%
2.....	12	16	34	67
8.....	14	13	11	17
16.....	10	15	4
30.....	21	17	26	29

The experiments with ethyl butyrate are summarized in table 1. The filaments were centrifuged with an acceleration of $75.6\times$ gravity for five minutes.

In table 1 it will be noted that when filaments were immersed for two minutes in 0.50 per cent ethyl butyrate and then centrifuged in ethyl butyrate, the chloroplasts were displaced in cells of 67 per cent of the filaments and not displaced in any cells of 33 per cent of the filaments, whereas in the controls the chloroplasts were displaced in cells of only 12 per cent of the filaments. In general it is apparent that the initial effect of 0.25 and 0.50 per cent ethyl butyrate was to decrease the value of c_0 , which is interpreted as a decrease in elasticity. Following the initial decrease, c_0 returned to the normal value. Unless the difference between the control (those in 0 per cent) and the anesthetized filaments in this or subsequent experiments was at least 10 per cent, the difference was not considered significant.

Results similar to those recorded in table 1 were obtained when concentrations of 1, 2, and 4 per cent ethyl acetate and when concentrations of 0.125, 0.25, and 0.50 per cent chloral hydrate were used. Again the greatest effects were obtained with the highest concentrations, 4 per cent ethyl acetate and 0.50 per cent chloral hydrate.

TABLE 2
EFFECT OF CHLOROFORM ON VALUE OF c_0

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF CHLOROFORM			
	0%	0.062%	0.125%	0.25%
2.....	1	0	13	100
4.....	0	1	4	76
8.....	1	0	2	88
12.....	5	3	60	86
20.....	14	15	11	41

TABLE 3
EFFECT OF ETHER ON VALUE OF c_0

MINUTES IMMERSED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN CONCENTRATIONS OF ETHER			
	0%	1%	2%	4%
1.....	16	16	30
4.....	14	4	4	25
6.....	7	8	7	8
26.....	17	15	19	20

The data for the experiments with chloroform are recorded in table 2. The filaments were centrifuged for five minutes with an acceleration of $75.6\times$ gravity.

The highest two concentrations of chloroform caused an initial decrease in the value of c_0 . With the highest concentration the value of c_0 was less than normal after twenty minutes' immersion.

The data for the experiments with ether are recorded in table 3. The filaments were centrifuged with an acceleration of $75.6\times$ gravity for five minutes.

When the filaments were immersed in 4 per cent ether for one minute prior to centrifugation, c_0 was slightly less than normal. With longer immersions it returned to approximately the normal value.

The data for the experiments with ethyl, n propyl, n butyl, and n amyl alcohols are recorded in table 4.

It will be noted (table 4) that in untreated filaments the average value of c_0 varies from day to day. At the time the experiments with

TABLE 4
EFFECTS OF ETHYL, n PROPYL, n BUTYL, AND n AMYL
ALCOHOLS ON VALUE OF c_0

MINUTES IMMERSED	CENTRIFUGAL ACCELE- RATION (\times GRAVITY)	MINUTES CENTRI- FUGED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED WHEN IMMERSSED IN				
			WATER	ETHYL	PROPYL	BUTYL	AMYL
			14 5% 6 2% 2 55% 1%				
3.	75 6	5	5	84	78	85	90
8.	75 6	5	26	15	56	52	19
16.	108.8	4	94	41	92	92	96
40.	108.8	4	97	0	2	83	83
			7.2% 3.1% 1.28% 0.5%				
3.	42.5	6	5	54	91	90	24
8.	42.5	6	4	8	40	71	17
16.	42.5	6	2	0	5	65
40.	108.8	4	90	51	93	93	96

the higher concentrations were performed c_0 was equal to or less than $75.6 \times$ gravity for at least 74 per cent of the filaments, whereas when the experiments with the lower concentrations were performed c_0 was equal to or less than $42.5 \times$ gravity for about 95 per cent of the filaments.

The alcohols in the concentrations used initially decreased the value of c_0 , thus indicating a decrease in elasticity and a likely change in the structure of the protoplasm. Following the initial decrease in protoplasmic elasticity, the elasticity became greater than

normal, as evidenced by an increase in the value of c_0 , when 14.5 and 7.2 per cent ethyl alcohol, 6.2 per cent propyl alcohol, 2.55 per cent butyl alcohol, and 1 per cent amyl alcohol were used. However, when the lower concentrations of propyl, butyl, and amyl alcohols were used the elasticity returned to approximately the normal value following forty minutes' immersion.

The following examples illustrate why the chloroplasts are displaced in a greater percentage of filaments which have been immersed for a short time in anesthetics than in those which have not been immersed in anesthetics. The data obtained when 14.5 per cent ethyl alcohol was allowed to act for three minutes will be used as an example. Assume for convenience that k equals 1 in the equation $V = k(c - c_0)$. The velocity of chloroplastic movement was zero for between 74 and 95 per cent (control was quite variable) of the untreated filaments. Hence for about 74 per cent of the filaments the normal value of c_0 was equal to or greater than $75.6 \times$ gravity but less than $108.8 \times$ gravity for about 95 per cent of the filaments (note percentage displacement when acceleration of $108.8 \times$ gravity was allowed to act on filaments in water). Hence for about 74 per cent of the filaments:

$$V = 1(75.6 - 75.6) = 0$$

or:

$$V = 1(75.6 - >75.6) = <0.$$

A velocity less than zero is impossible. Following three minutes' immersion in 14.5 per cent ethyl alcohol, the average value of c_0 had been decreased. Hence for 84 per cent of the filaments:

$$V = 1(75.6 - <75.6) = >0.$$

The chloroplasts will move in 84 per cent of the filaments.

It is believed that stimuli which alter the value of c_0 cause changes in the structure of *Spirogyra* protoplasm. The nature of the prob-

able changes is not known. It may be that the orientation of the long interlaced molecules or micelles is changed or that the molecules or micelles alter their shape. The hypothetical diagrams shown in figure 1 represent one of several possible alterations in structure following short immersions in some anesthetics of the proper concentration. Diagram *a* represents normal protoplasm with a ball suspended in it. The ball will move only when an initial shearing stress, c_0 , is exceeded. Diagram *b* represents protoplasm after two

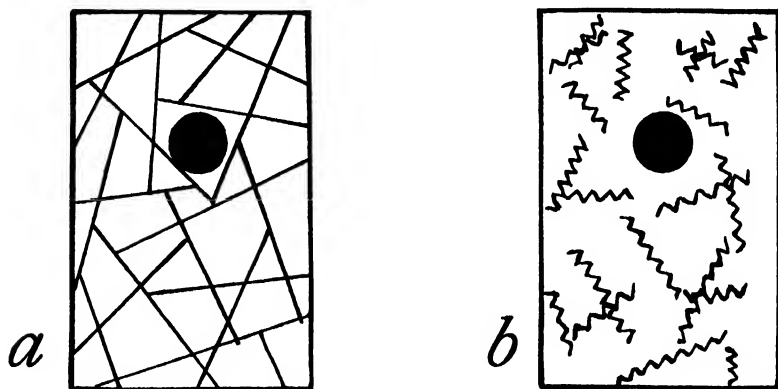


FIG. 1

minutes' immersion in one of the anesthetics used. The shearing stress necessary to cause any movement of the ball has been decreased.

Summary

1. Filaments of *Spirogyra* which had been immersed for different periods of time in several concentrations of various anesthetics were centrifuged with accelerations which usually caused no displacement of the chloroplasts in most cells of non-anesthetized filaments.

2. The initial effect of certain concentrations of the anesthetics was to decrease the value of c_0 in the equation, $V = k(c - c_0)$, which approximately governs the rate with which the chloroplasts in cells of *Spirogyra* move in response to centrifugal accelerations. A decrease in c_0 was interpreted as a decrease in protoplasmic elasticity.

3. With longer immersions c_0 returned to approximately the normal value except in the case of the alcohols, in which instances c_0 became greater than normal (elasticity increased).

I am deeply grateful to my wife and to Mr. RALPH AMES for aid in obtaining data.

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EFFECTIVENESS OF PHOTOPERIODIC TREATMENTS OF PLANTS OF DIFFERENT AGE

H. A. BORTHWICK¹ AND M. W. PARKER²

It has been reported by CAJLACHJAN (2) that short day treatments applied to millet were most effective when the plants were one and five weeks old, and that it was at these periods that millet made its most rapid growth.

In the present investigation the Biloxi soy bean, which is very responsive photoperiodically, was used. Critical morphological examinations were made of the fresh material before and after short day treatments. It is unfortunate that many investigators in this field have failed to examine their material critically; thus the results are difficult to interpret. Six plantings of about 150 plants each were made in the greenhouse at weekly intervals from January 21 until February 25, 1938. All plants were grown at the intensity and duration of natural light then prevailing, supplemented by approximately 50 foot candles of Mazda light from sundown until midnight.

From among the 150 plants of each planting, 72 of the most uniform in size and appearance were again selected. These were then divided into six lots of twelve plants each. Beginning one week after planting, one lot was subjected to an induction treatment of four 8-hour days and then returned to a 16-hour day to continue development. At the end of the second week another lot was similarly treated, and this procedure was continued until all six lots had been similarly disposed of. The last lot received its induction treatment six weeks after planting. Except for the induction periods of four 8-hour days, none of the plants was subjected to less than a 16-hour day.

Two weeks after the beginning of the induction treatment, six plants from each lot were dissected and examined in detail to de-

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termine the number and location of flower buds. Three weeks later the remaining plants of each lot were similarly examined.

It has previously been shown (1) that the first response of the Biloxi soy bean to a short photoperiod occurred in the axil of the leaf that was approximately third from the tip of the main axis when the treatment was started. Similar response also occurred simultaneously approximately three nodes from the tips of certain axillary buds. In the experiment here reported reference is made only to flower primordia initiated on the main axis.

If treatment with short photoperiod is continued for many days, flower primordia are eventually initiated at all the nodes above the point where the first flower buds were found. With treatments of only four 8-hour days, however, flower primordia may arise at a few nodes; but under the influence of 16-hour days following the induction period there is an increasing tendency toward differentiation of shoots instead of flower primordia at the newly forming nodes above. The number of nodes at which flower primordia are differentiated affords a measure of the effectiveness of identical treatments given to plants of different ages.

The number of plants forming flower primordia in response to identical photoperiodic treatments increased with the age of the plant (table 1). No plants responded when treated one week after planting, and only nine out of seventy-two showed any response when two weeks old. Most of the 3- and 4-weeks-old plants and all of the 5- and 6-weeks-old ones formed flower primordia under similar treatment.

The treatments at one date constitute an exception to this relationship. The 3-weeks-old lot of planting series 5 and the 4-weeks-old lot of planting series 4, which were given short day treatment beginning March 11, failed to make visible response. All the 5-weeks-old plants of lot 3 and the 6-weeks-old ones of lot 2 treated at the same time, however, produced flower primordia. The plants of all these lots were kept together and consequently received exactly the same treatment. The difference in their response is unexplained but is apparently correlated with their age or size. At the time this particular treatment was given, the night temperature of the green-

house was about 5° higher than was true with the other treatments, and the light intensity was very low because of stormy weather.

TABLE 1
NUMBER OF PLANTS IN EACH TREATMENT SHOWING FLOWER
BUD FORMATION IN RESPONSE TO INDUCTION PERIOD

PLANTING SERIES	AGE AT TIME INDUCTION TREATMENTS WERE STARTED (WEEKS)					
	1	2	3	4	5	6
1.	0	3	10	10	12	12
2.	0	2	11	12	12	12
3.	0	0	12	12	12	12
4.	0	3	11	0	12	12
5.	0	1	0	12	12	12
6.	0	0	12	12	12	12
Total. . .	0	9	56	58	72	72

TABLE 2

AGE AT START OF INDUCTION PERIOD (WEEKS)	TOTAL NUMBER OF NODES PER PLANT AT WHICH FLOWER PRIMOR- DIA WERE INITIATED		TOTAL NODES PRESENT PER PLANT AT START OF INDUC- TION PERIOD	LOCATION OF FLOWER PRIMORDIA (NODES FROM BASE OF PLANT)			
				FIRST FLOWER PRIMORDIA FORMED		LAST FLOWER PRIMORDIA FORMED	
	AT END OF 2 WEEKS*	AT END OF 5 WEEKS		AT END OF 2 WEEKS	AT END OF 5 WEEKS	AT END OF 2 WEEKS	AT END OF 5 WEEKS
1.	0	0					
2.	1.1	1 0		6.7	7 0	6 8	7 0
3.	1 8	1 7	10	8 8	8 6	0 6	0 3
4.	2 3	2.1	12.5	10.5	10 5	11 8	11 6
5.	2 0	2 8	14 9	11.8	12.2	13 7	14 0
6.	4.1	4 4	16.6	13 4	13 6	16 5	17 0

* Time measured from beginning of induction period.

Flower primordia were produced at more nodes per plant on the older than on the younger plants (table 2). Evidently the stimulus of four 8-hour days resulting in differentiation of flower buds has a

greater effect if it is received after the plants have attained greater age and size. The development of all flower primordia was apparently well advanced within two weeks after the start of treatment, for there was no significant difference in the number found then and three weeks later (table 2).

Comparison of the figures in table 2 showing the position of the uppermost flower primordia with those showing the total number of nodes present at the beginning of treatment indicates that flower primordia were initiated in the axils of leaves already present at the time the treatments were begun. One possible exception is in the 6-weeks-old lots. Although this suggests that there is no "carry-over" effect of an induction period to structures differentiated subsequent to it, later experiments made during the summer show that there is such an effect. In these later experiments flower primordia have been found at positions several nodes above the node which was the uppermost one when the induction period was terminated.

The results of this experiment show that the effectiveness of a photoperiodic treatment consisting of four 8-hour days increases with the age of the plant to which it is applied until the plants are at least six weeks old. It would be desirable to investigate the responses of still older plants. The complete failure on one occasion of 3- and 4-weeks-old plants to respond to the same treatment that resulted in flower bud initiation in 5- and 6-weeks-old plants indicates that other environmental factors may influence the photoperiodic response. Data which will be presented at another time show that the intensity of illumination during treatment exercises a controlling influence on the response. Temperature, humidity, and other factors also play a part, and under conditions of very brief photoperiodic treatment these factors other than the length of the period of illumination may become limiting.

The reason for differences in effectiveness of photoperiodic treatments on plants of different ages is probably associated with differences in leaf area. CAJLACHJAN (3) has called attention to the fact that plants respond photoperiodically as soon as the first green leaf appears and not before. The data here presented are in agreement with this observation. Other data, which will be published later,

show definitely that the response of the Biloxi soy bean to photoperiodic treatment is mainly an expression of the stimuli to which the leaves have been subjected.

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A MICRO-KJELDAHL METHOD INCLUDING NITRATES

RUFUS H. MOORE

To those who find it necessary to use a micro-method for the determination of total nitrogen in the presence of nitrates, the following procedure is offered.

This procedure is an adaptation of the reduced iron macro-method of PUCHER, LEAVENWORTH, and VICKERY (3) to the micro-method described by PREGL (2). As the former requires 35 cc. of concentrated H_2SO_4 and the latter only 1 cc., the adaptation consists essentially of reducing all reagents employed, up to the point of adding the catalyst, to $1/35$ of that recommended by the authors of the reduced iron method.

The complete Eimer and Amend micro-Kjeldahl apparatus was used for the tests reported. In the modified procedure, from 10 to 50 mg. of powdered sample were used. To the sample in the digestion flask 1 cc. water, 0.29 cc. $(1 + 1)$ H_2SO_4 , and 0.086 (± 0.0086) gm. iron powder were added. The flask was shaken ten minutes and cooled slightly under tap water (if necessary) to prevent excessive frothing. The flask was then heated five minutes over a very low flame and allowed to cool. After adding 0.86 cc. concentrated H_2SO_4 , a knife point of a 1:3 mixture of K_2SO_4 and $CuSO_4$, and two small angular quartz pebbles, digestion over a medium flame was allowed to continue until fumes appeared. Nitrogen-free Superoxyl was added until the solution remained blue. After heating over a high flame for one hour, the flask was allowed to cool slightly and 1 cc. of water added. The sample was distilled as described by PREGL, a methyl red-methylene blue indicator having been used in titration.

Powdered samples were weighed on small pieces of cigarette paper, kept between paired watch glasses during weighing to prevent excessive absorption of moisture. The paper was folded over the sample in such a way that it would open readily when dropped into the bulb of the digestion flask and the latter shaken, thus dispersing the sample for rapid solution in the acid. The cigarette paper used

was free from nitrogen, digested readily, was sufficiently fine-pored to retain the sample, and flexible enough to permit easy handling.

TABLE 1
SUMMARY OF PRECISION TESTS OF REDUCED
IRON MICRO-KJELDAHL METHOD

MATERIAL TESTED	NO. OF TRIALS	0.01 N EQUIVALENT OF SAMPLE (CC.)	NITROGEN FOUND (MG.)
Blank.....	6	0.351 ± 0.0056	0.049
NaNO ₃	5	1.079 ± 0.0096	0.151
Witte's peptone.....	3	2.524 ± 0.0067	0.359
NaNO ₃ and Witte's peptone..	3	3.627 ± 0.0044	0.514

TABLE 2
TOTAL NITROGEN IN PEANUT TISSUES AS DETERMINED
BY REDUCED IRON MICRO-KJELDAHL METHOD

PLANT FRACTION	WEIGHT OF SAMPLE (MG.)	TOTAL NITROGEN (%)	DEVIATION FROM MEAN (%)
Primary root and hypocotyl..	24.78	0.690	± 1.71
	21.32	0.714	
Green gynophores.	27.44	2.266	± 1.52
	18.31	2.336	
Mature gynophores	26.23	1.980	± 0.69
	25.57	1.953	
Mature gynophores	26.93	1.382	± 0.50
	23.17	1.396	
Immature fruits....	12.59	3.907	± 1.24
	10.75	4.005	
Immature fruits.	11.31	5.362	± 0.00
	15.26	5.362	
Mature seeds.	45.88	2.945	± 0.62
	52.64	2.982	

For determination of nitrogen in alcoholic extracts 1 cc. aliquots should be used. Iron powder was measured in a small aluminum spoon.

The precision of the foregoing adaptation was tested on a solution of NaNO_3 , Witte's peptone dissolved in 2 per cent H_2SO_4 , and a mixture of these two. As each sample of NaNO_3 was calculated to contain 0.1502 mg. of nitrogen and each of the peptone aliquots determined to contain 0.3592 mg. of nitrogen, 29.5 per cent of the nitrogen in the combined sample was nitrate nitrogen. Sufficient distilled water was added in each case to bring the volume of the sample to 1 cc. before reagents were added. Results are condensed in table 1. The average recovery of nitrogen from the NaNO_3 samples was calculated as 100.64 per cent, and from the combined samples of NaNO_3 and Witte's peptone as 100.85 per cent. More extensive tests of the precision of this method are to be desired.

The method was used on certain oven-dried fractions of the peanut plant, which provided too small an amount of tissue for macro-analysis. Results are given in table 2. This method was also used by JONES (1) to determine total nitrogen in the presence of nitrates in wheat seedlings.

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2. PREGL, FRITZ, Quantitative organic microanalysis. Philadelphia. 1930.
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CURRENT LITERATURE

General Plant Physiology. By E. C. BARTON WRIGHT. London: Williams and Norgate Ltd., 1938. Pp. 539.

A new textbook of plant physiology from an English plant physiologist will be read with much interest by American students of the subject. The author is well known by reason of his *Recent Advances in Plant Physiology*, which appeared in two editions, 1930 and 1933. The new work is elementary, intended for first and second year students in colleges and universities. The material is presented in three parts: general physiology of the cell; metabolism; and growth, reproduction, and irritability.

The first part includes chapters on the scope of physiology, colloids and protoplasm, osmotic pressure and the water relations of the plant, permeability, transpiration, and the ascent of sap. The metabolism section offers chapters on catalysis and enzymes, photosynthesis, the fats, nitrogen metabolism, ash, the transport of solutes, and respiration. The final section includes germination, growth, reproduction, and irritability and plant movements. A brief appendix discusses pH, following which there is a bibliography of 87 titles, mainly texts or monographs, and author and subject indexes.

Readers should keep in mind the great difficulty confronting anyone who writes a text on a subject which advances as rapidly as plant physiology has during the last fifteen years. Before any text can be published, some of the material is almost certain to be superseded by newer work. The foreword by F. GOWLAND HOPKINS calls attention to the logical arrangement of the presentation, and to the relative independence of the sections, so that individual chapters may be read with satisfaction. English plant physiologists have contributed much to the development of our knowledge, and American students should be given an opportunity to read and appreciate this interesting and very readable text.—C. A. SHULL.

General Physiology. By PHILIP H. MITCHELL. New York: McGraw-Hill Book Co., 1938. Pp. xviii+853. \$6.00.

Laboratory Manual of General Physiology. By PHILIP H. MITCHELL and IVON R. TAYLOR. New York: McGraw-Hill Book Co., 1938. Pp. xv+142. \$1.50.

The third edition of MITCHELL's *General Physiology* is now accompanied by a useful laboratory manual, designed to offer choice for a year of laboratory study of physiological problems. The text has been carefully revised, with extensive changes in the chapters dealing with excitation, contraction, transmis-

sion, membrane structure, cell permeability, physiological oxidations, muscle chemistry, vitamins, and hormones. These changes have been dictated by the rapid developments in these fields. New sections deal with organizers or evocators, oxidation reduction potentials, Liesegang phenomena, and the physiological study of temperature characteristics. The discussion brings to the student a knowledge of the improved techniques which have been developed since the second edition appeared, and the new theories of physiological action are given adequate attention for a work designed for a single course in general physiology. Some physiologists may criticize the choice of material, but it attempts to present some phases of plant physiology while placing the main emphasis on animal physiology.

The laboratory manual follows the order of presentation of the text, and offers a wide choice of materials. The text contains twenty-five chapters, and twenty-one of these are represented in the manual. There are 254 laboratory exercises, many more than can be used in a full year course. Instructors may thus adapt the work to individual laboratories by choice in accordance with equipment, or in accordance with the emphasis desired. The reviewer finds them interesting and valuable. Teachers will surely welcome the addition of the laboratory manual, which should make the use of the text feasible in any institution which possesses good laboratory equipment.—C. A. SHULL.

Cryptogamic Botany: Vol. I. Algae and Fungi. By GILBERT M. SMITH. New York: McGraw-Hill Book Co., 1938. Pp. vi+545. Figs. 299. \$4.00.

As stated in the preface, "this book is designed for students who have had an introductory course in botany and who wish to make a more detailed study of plants below the level of the seed plants. It is written from the standpoint that a thorough knowledge of a representative series in each of the major groups is better than scraps of information about a large number." With this purpose in mind, the author has brought together representative examples of the various divisions and classes of the algae and the fungi.

Following chapter I, which discusses the classification of spore-producing plants, some 338 pages are devoted to the algae and 172 to the fungi. The algae are presented under seven divisions: Chlorophyta or grass-green algae, Euglenophyta or euglenoids; Pyrrophyta, which includes cryptomonads and the dinoflagellates; Chrysophyta, which includes the yellow-green algae (Xanthophyceae), the golden-brown algae (Chrysophyceae), and the diatoms; Phaeophyta or brown algae; Cyanophyta or blue-green algae; and the Rhodophyta or red algae. The fungi are presented under two divisions, Myxothallophyta or slime molds and Euimycetae, or true fungi, embracing the classes of Phycomycetes, Ascomycetes, Basidiomycetes, and the Fungi Imperfecti.

The examples used throughout the text have been carefully chosen, and the details of their life cycles have been clearly and, in the main, completely covered.

The illustrations are largely original, are judiciously selected, and exceptionally well executed. Both the author and the publisher are to be commended for their work.

A feature which merits comment is that this is the first attempt which has been made to bring together in one volume a fairly condensed treatment of the principal groups of the algae, and thus make available to English speaking students the essential features of the life histories of the representatives chosen. This is a noteworthy achievement, and would of itself make the book a real contribution.

Some botanists may think that too little space has been given to the fungi, but the reviewer is of the opinion that the author has succeeded in accomplishing his purpose of adequately presenting selected representative forms covering the principal phylogenetic tendencies and the significant types of reproductive structures, including the nuclear phases. The book is suitable for a semester course covering the algae and the fungi, or for two quarter courses devoted to each group separately.—J. M. BEAL.

Cryptogamic Botany: Vol. II. Bryophytes and Pteridophytes. By GILBERT M. SMITH. New York: McGraw-Hill Book Co., 1938. Pp. vii+380. Figs. 224. \$3.00.

The author has brought together the essentials of the present day knowledge about bryophytes and pteridophytes in a simple, direct manner that will make it usable both for class work and for reference. While the text is necessarily abbreviated, the bibliographies are cited in such a manner that the study may be greatly expanded when desired. Illustrations are ample and well chosen. In general they are well described and lettered.

The subject matter is consistently arranged according to taxonomic classification. The distinguishing characteristics of each class, order, and family are discussed. In each family the distribution, structure, and life histories of representative genera are described. The first 113 pages are devoted to the Bryophyta, while 253 pages deal with the Pteridophyta, both fossil and living.

In the Bryophyta the Anthocerotae are regarded as a group of equal rank with the Hepaticae and Musci. The Pteridophyta are arranged in four large groups, the Psilopytinae, Lycopodinae, Equisetinae, and Filicinae.

In discussing the origin of the Pteridophyta, the author recognizes the present tendency to derive the group from algal ancestors. He, however, presents a convincing discussion of its possible origin from ancestors of the anthocerotean type. Theories as to the origin of the vascular system and its evolution among Pteridophyta constitute a valuable and interesting part of the introduction to this group.

This book will fill a real need for a textbook on the archegoniates.—ELDA R. WALKER.

Manual of Pteridology. Edited by FR. VERDOORN. The Hague: Martinus Nijhoff, 1938. Pp. xv+640. Illustrated. 24 guilders.

This is a collection of essays by twenty specialists on various subjects dealing with pteridophytes. It is intended primarily to broaden the background of taxonomists but it will be of equal value to those interested in ferns from other viewpoints. There is scarcely a phase of information in connection with the group that is not reviewed.

The foreword by F. O. BOWER is followed by chapters on morphology, anatomy, experimental morphology, cytology, genetics, growth and tropisms, chemistry, ecology, geography, pathology, symbiotism both with algae and with mycorrhizal fungi, gall formations, and phylogeny. About half of the chapters are in English; the remainder in German. By means of footnotes and bibliographies much literature is made available.

The volume is a most valuable compendium of information along all lines dealing with pteridophytes, both living and fossil.—ELDA R. WALKER.

THE BOTANICAL GAZETTE

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EMBRYOLOGY OF CERTAIN RANALES

T. T. EARLE

(WITH EIGHTEEN FIGURES)

Studies in the embryology of various members of the Ranales have been made from time to time, but no one seems to have called attention to the similarities and dissimilarities that occur in the group as a whole. Of the five families that have been investigated, it is of interest to note that the embryology of the Magnoliaceae, Ranunculaceae, and Berberidaceae conforms to one general plan which is quite different in certain respects from another rather uniform plan found in the Nymphaeaceae and Ceratophyllaceae. The present investigation of the embryology of *Magnolia grandiflora* L. and *Cimicifuga racemosa* (L.) Nutt. furnishes additional evidence as to the uniformity of embryo development in the first three families.

Investigation

MAGNOLIA GRANDIFLORA

Magnolia grandiflora is native to the coastal plain of the southeastern United States, but is successful in cultivation farther inland. Material examined for this paper was collected at Greenville, South Carolina, at intervals from the first of May until the latter part of September, in 1933. In the northwestern part of South Carolina, where Greenville is situated, flowering begins about the first of May and newly opened flowers may be found as late as the end of July, so that ovules in various stages of development may be collected simultaneously.

The many unicarpellate pistils of a flower, arranged spirally upon a cone-shaped receptacle, each contain two anatropous ovules, one or both of which may develop into a seed. Each pistil develops into a small follicle at maturity, and the aggregation of follicles on the receptacle forms a conelike structure about 3 to 5 inches in length

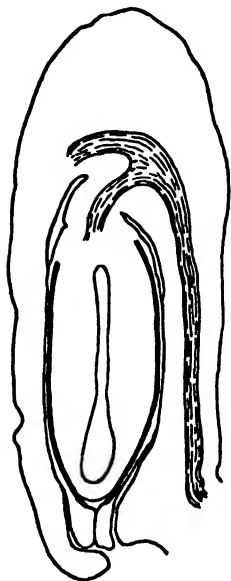


FIG. 1.—Median longitudinal section of ovule of *Magnolia grandiflora* at time of flowering.

and about 2 to 2.5 inches in diameter. When mature, the follicles dehisce and allow the seeds to escape, although for a time they remain attached to the follicle by means of a slender strand of protoxylem, the remains of the funiculus. This eventually breaks and the seed falls to the ground; or it sometimes happens that the entire cone with the attached seeds is shed.

OVULE AND EMBRYO SAC.—Development of the ovule in this plant is essentially the same as that in *M. umbrella* Desr. [*M. tripetala* L.] described by GRAY (9). Figure 1 is a longitudinal section of an ovule of *M. grandiflora* at the time of opening of the flower bud. The single vascular strand of the ovule is seen to pass unbranched throughout the entire length of the raphe, and then ends in the chalazal region of the ovule in short branches, one of which is directed toward the embryo sac. This condition is retained during the entire development of the seed.

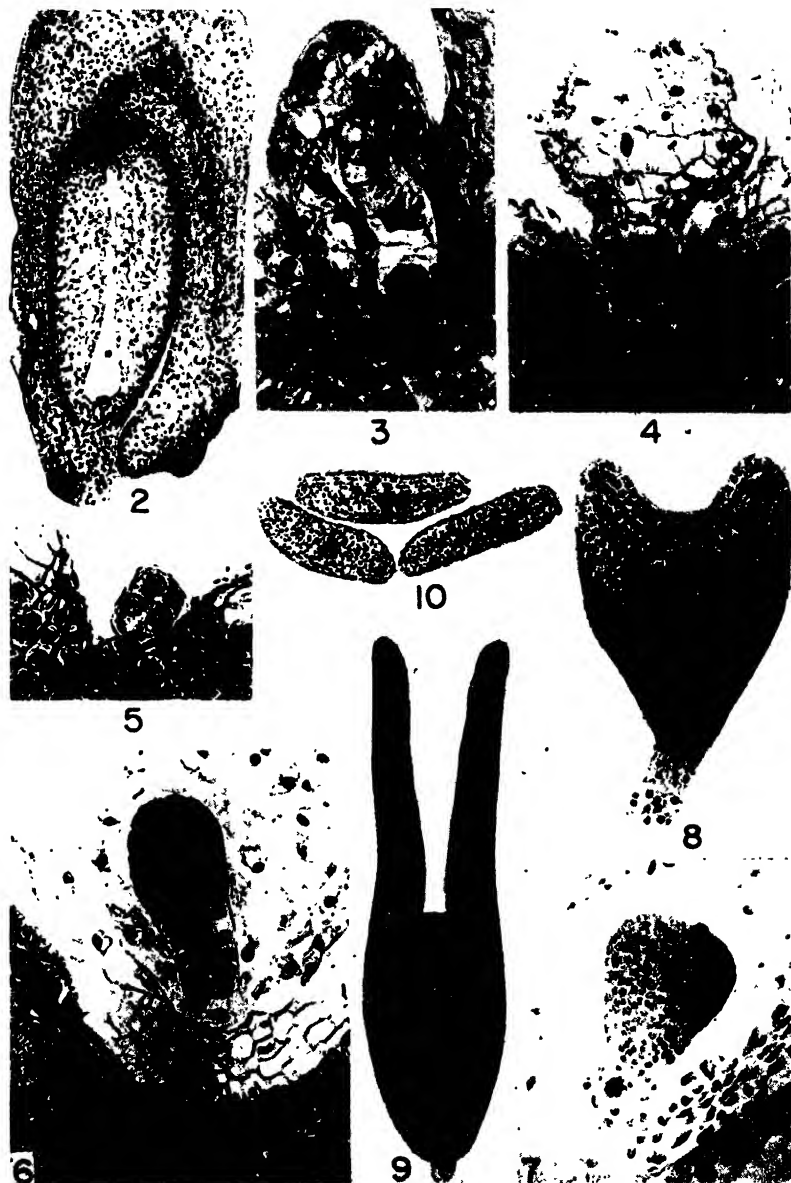
The mature embryo sac is long and slender, but enlarges into a club-shaped expansion at the micropylar end and also enlarges slightly at the chalazal end. It is extremely difficult to fix properly owing to the fact that it is surrounded by a considerable mass of nucellar tissue. It is circular in cross section, measuring about 110μ in diameter at the largest portion of the club-shaped expansion, about 35μ in diameter at the chalazal end, and is approximately 650μ in length. The three antipodal nuclei are situated at the extreme base of the sac, the two polar nuclei occupy a position about one-fourth the length of the sac from the egg apparatus, while the egg and two synergids are at the apex of the sac.

ENDOSPERM.—The endosperm nucleus, either before or after fusion with the sperm nucleus, moves to a position even closer to the egg apparatus, and it is in this region that the endosperm starts its development. The early divisions of the endosperm nucleus are very rapid and the number of free nuclei formed is comparatively small. They occupy only the expanded end of the embryo sac. Wall formation occurs shortly after the appearance of these free nuclei and the endosperm appears as a globular mass of tissue, almost obscuring the fertilized egg (fig. 3). At this time the ovule begins to increase in size and the nucellus begins to disorganize, especially in the region of the growing endosperm. At first the endosperm is a rather compact mass of tissue (figs. 3, 4), the cells being thin-walled and relatively large. About three or four weeks after the external flower parts have fallen away the endosperm enlarges enormously, the cells becoming very much larger, and the tissue resorbing all of the nucellus except the small mass on which the embryo rests and a thin sheet immediately under the inner integument. At this time the endosperm is very watery and extremely difficult to fix on a slide. In material collected about the middle of August, however, the endosperm is firm in texture and is beginning to assume the character of the endosperm of the mature seed.

According to EVANS (8), the cells of the endosperm of the mature seed are rich in protein reserve bodies and small globules of oil, but contain no starch.

EMBRYO.—The fertilized egg remains dormant for a considerable length of time while the endosperm is developing rapidly. Ovules collected about two weeks after the flower had opened show the fertilized egg undivided, while the endosperm is a considerable mass of tissue (fig. 3). An embryo in the two-celled stage was not observed, but a three-celled embryo is shown in figure 4. The two lower cells of this embryo are in contact with the nucellus, while the third cell, which is larger than either of the two lower ones, is on top of them. It is obvious, therefore, that the first division is transverse and the second division is in the basal nucleus and perpendicular to the first. The somewhat later division of the upper nucleus is also perpendicular to the first division, and an almost spherical four-celled embryo results (fig. 5).

The divisions immediately following are comparatively slow and



FIGS. 2-10.—*Magnolia grandiflora*. Fig. 2, median longitudinal section of ovule at time of flowering. Fig. 3, fertilized egg, cellular endosperm, and partially resorbed nucellus. Fig. 4, three-celled embryo and cellular endosperm. Fig. 5, four-celled embryo. Fig. 6, club-shaped embryo showing more vacuolated suspensor cells. Fig. 7, flat-topped embryo. Fig. 8, slightly older embryo showing primordia of cotyledons. Fig. 9, longitudinal section of mature embryo. Fig. 10, transection through cotyledons of tricotyledonous embryo.

are probably not so uniform in their direction. About four weeks after the first division of the fertilized egg, which is about six weeks after pollination, the embryo is a slightly club-shaped mass of entirely undifferentiated cells, about ten cells high and several cells in thickness throughout.

Cell divisions become more frequent at this time, especially in the upper half of the embryo, and this portion bulges slightly in all directions, becoming hemispherical on the top and sides and tapering gradually into the massive stalk below (fig. 6). This stalk becomes a massive suspensor several cells in thickness. Cell divisions continue to be very frequent in the embryo and may be seen to take place in all planes, the whole structure becoming considerably larger, although still retaining the same shape. As yet there is no specialization of any of the cells in the body of the embryo, all of them being about the same size and containing large, spherical nuclei and dense cytoplasm. In the part that is to become a suspensor, however, the cells are somewhat larger and the cytoplasm less dense.

Material fixed about the middle of August shows a flattening of the distal end of the embryo (fig. 7). This condition is not retained long, for soon the rudiments of the cotyledons make their appearance (fig. 8). Both start at the same time and are exactly the same size. Embryos which were dissected out and examined showed no indication of a crescentic ridge from which the cotyledons might arise, but each cotyledon was seen to be an independent structure from the very beginning of its existence. They grow rather rapidly and it is a matter of only a few days until they are fairly elongated structures. Occasional embryos with three cotyledons were found, in which case the three cotyledons are equidistant from one another as in the normal type with two cotyledons.

While the cotyledons are growing both in length and thickness, numerous cell divisions may be seen in the body of the embryo in all planes. At the same time, the primordial cells of the primary vascular tissue are elongating, strands passing into each cotyledon from the hypocotyl. Growth in the whole embryo is rapid from this time on, although the cells in the massive suspensor have ceased dividing. Before the embryo has reached its full length the radicle may be distinguished and the plerome is well established, traversing the entire distance from radicle to cotyledons.

The embryo (fig. 9) is mature by about the middle of September. It is approximately 1 mm. in length. The plerome of the hypocotyl sends three branches into each cotyledon, and these are connected at various intervals by anastomosing strands of elongated cells. In cross section the cotyledons are slightly curved, their concave surfaces facing each other. The distal end of the hypocotyl between the cotyledons is flat in most cases but sometimes bulges slightly, and the protoplasm of the cells in this region is more compact than in the other cells of the embryo. The embryo remains attached to the massive suspensor, which still rests on the remains of the nucellus at the micropylar end of the seed. A root cap is distinguishable between the suspensor and the growing point of the radicle.

CIMICIFUGA RACEMOSA

Cimicifuga racemosa grows in rich woods throughout the eastern United States from Maine to Georgia, and as far west as Wisconsin. It reaches an altitude up to 4000 feet in the mountains of western North Carolina. Material was collected during the months of June, July, and August in 1934 and 1935 in Greenville County, South Carolina, and Brevard County, North Carolina.

The plants are rather conspicuous, with large, ternately compound leaves and long flowering stems, up to 10 feet in height. The inflorescence is a raceme up to 2 feet in length. The flowers consist of four or five petaloid sepals which fall away soon after the flower opens, several small clawed petals, numerous stamens, and usually a single pistil, although occasionally flowers with two or three pistils have been found. Flowering takes place from May to August in North and South Carolina, depending upon the altitude at which the plants are growing. The fruit is a follicle containing eight to twelve flattened seeds packed in two horizontal rows in the pod.

OVULE AND EMBRYO SAC.—The anatropous ovules lie in two horizontal rows in the pistil. Sections fixed about three weeks before the opening of the flower bud show the primordia of a pair of ovules as two protuberances of homogeneous tissue, directed obliquely from each other (fig. 11A). Unequal growth causes the ovular primordia to bend, and at the same time the inner integument makes its appearance. It appears as a collar-like ridge, de-

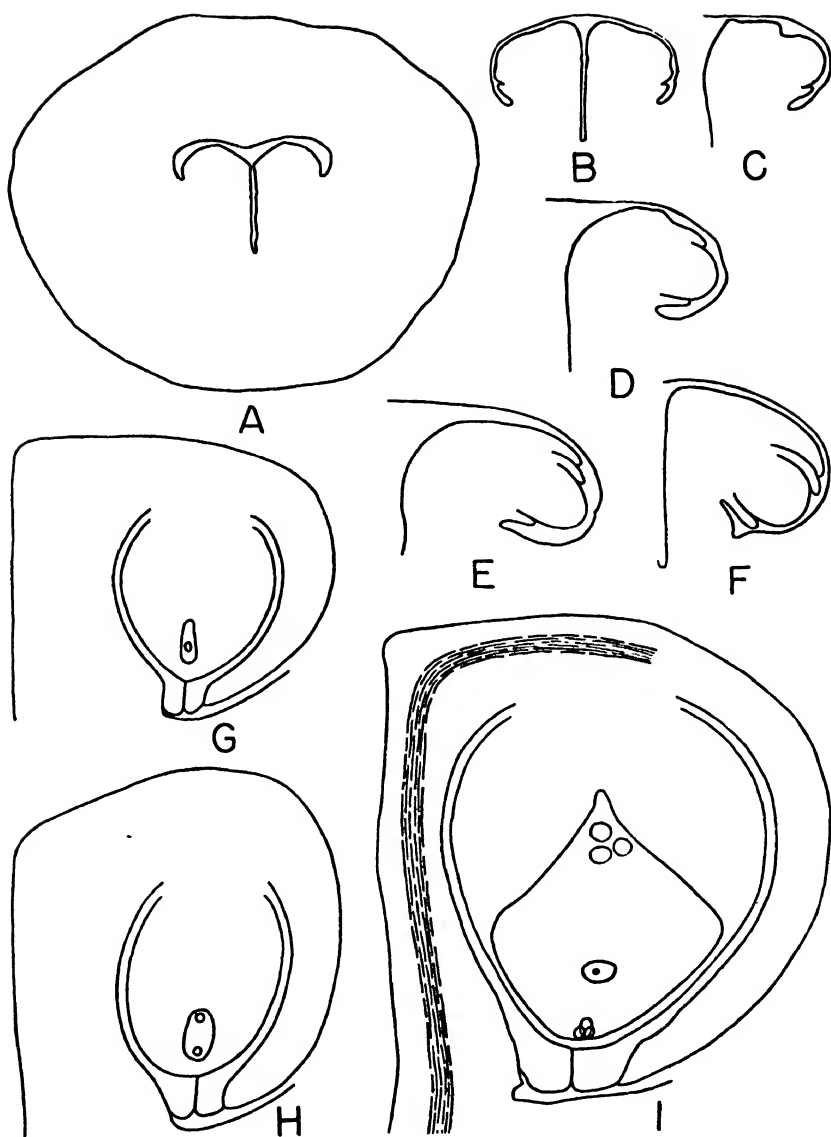
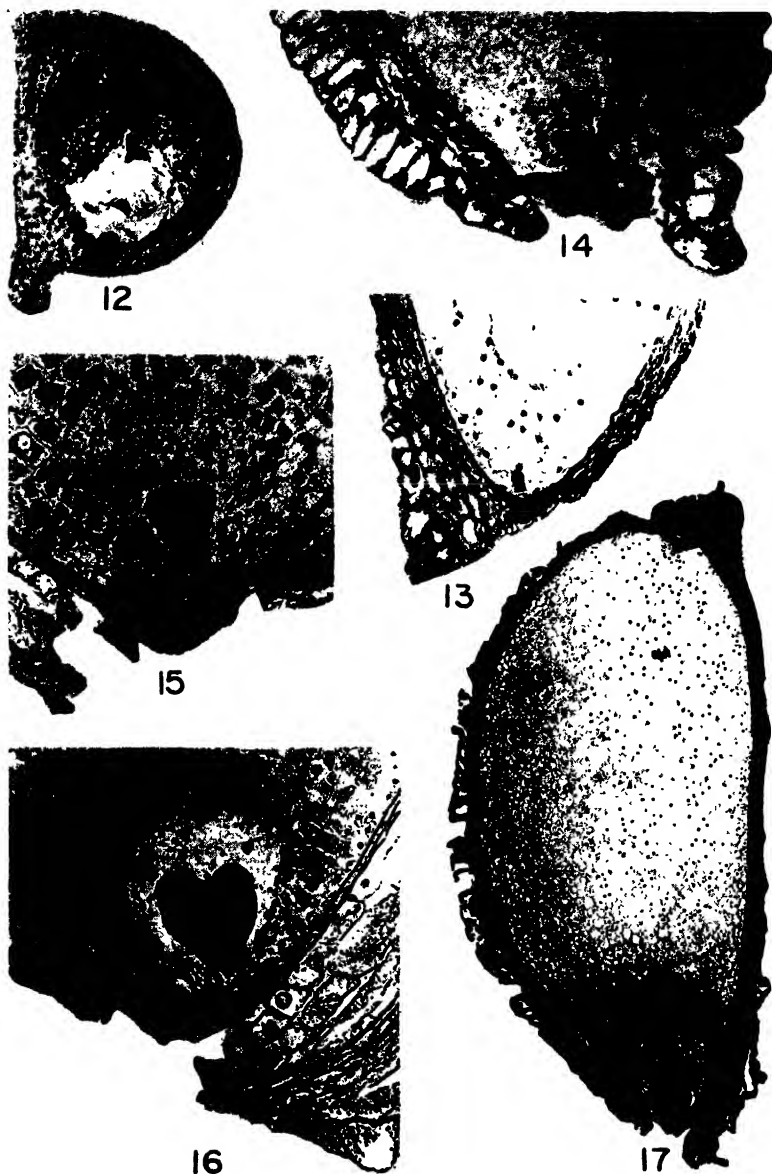


FIG. 11.—A-I. Development of anatropous ovule of *Cimicifuga racemosa*

veloping first on the under side of the nucellus (fig. 11*B*) and finally completely encircling it (fig. 11*C*, *D*). It is two layers of cells in thickness at the beginning and retains this condition during its entire existence, except in the part that surrounds the micropyle. Shortly after the appearance of the inner integument, the outer integument appears (fig. 11*D*, *E*). It is also two layers of cells in thickness at first but soon begins to thicken as a result of tangential divisions. The ovule continues to bend until finally it becomes entirely inverted (fig. 11*F*, *G*), during which time the inner integument grows completely down over the nucellus, forming the micropyle. In the micropylar region the inner integument becomes several layers of cells thick. The outer integument never quite reaches the micropyle. When the ovule is mature there is a single vascular strand traversing the raphe and ending blindly in the chalazal region (fig. 11*I*).

The mature embryo sac is practically rhombic in shape as seen in longitudinal section (figs. 11*I*, 12). One angle is situated at the micropylar end of the ovule and from the opposite angle there is a slight projection of the embryo sac into the nucellus. At the base of the sac are three prominent antipodal nuclei, a little above the center is a large fusion nucleus, and at the micropylar end is the egg apparatus, consisting of an egg and two synergids. At its greatest length the embryo sac is about 300 μ , and approximately 250 μ at its greatest width.

ENDOSPERM.—Division of the primary endosperm nucleus occurs soon after fertilization. The nuclei of the endosperm undergo a period of repeated, simultaneous, free divisions, and eventually are distributed in the parietal layer of cytoplasm of the embryo sac, as a single layer. While this parietal layer of free nuclei is forming, the nucellus is rapidly resorbed and the ovule is increasing in size. The nuclei of the endosperm are much larger than those of the developing embryo, and each contains three to five nucleoli. When the embryo consists of about eight to ten cells the nuclei of the endosperm layer undergo a periclinal division, forming a second layer inside the first. Wall formation then occurs between the nuclei, not simultaneously, but beginning between the nuclei in the chalazal region and progressing toward the micropylar end. Additional peri-



FIGS. 12-17.—*Cimicifuga racemosa*. Fig. 12, median longisection of ovule at time of flowering. Fig. 13, undifferentiated embryo and endosperm that has not filled embryo sac. Fig. 14, slightly club-shaped embryo with vacuolated suspensor cells; endosperm completely filling embryo sac. Fig. 15, flat-topped embryo surrounded by partially digested endosperm. Fig. 16, slightly older embryo showing primordia of cotyledons. Fig. 17, median longisection of mature seed showing embryo surrounded by partially digested endosperm.

clinal divisions take place in rapid succession and very soon the endosperm is a solid mass of tissue within the integuments, the nucellus having been completely resorbed. The endosperm is very watery at this time, but at about the time the club-shaped end of the embryo begins to enlarge (fig. 14) it is much firmer and the cells stain more heavily. Mitotic figures may still be seen in the part immediately surrounding the embryo. As the embryo enlarges the endosperm cells surrounding it appear to be partially digested. In the preparation of sections of ovules in later stages of development the walls of these partially digested endosperm cells do not stain with haematoxylin, thus giving the appearance of a cavity between the embryo and the undigested endosperm (figs. 15, 16). In sections stained with alcoholic safranin, however, the walls of these partially digested endosperm cells stain heavily, showing it to be a globular mass of tissue surrounding the embryo (fig. 17). The endosperm of the mature seed is oily in texture, the cells containing numerous small globules of oil.

EMBRYO.—The endosperm is still in the free nuclear condition when the first division of the fertilized egg occurs. The nucellus in the micropylar region of the ovule may be completely resorbed by the time the first division takes place, in which case the embryo is situated next to the inner integument, very close to the micropyle; or there may be a small amount of nucellar tissue still present between the young embryo and the integument. The direction of the first division of the fertilized egg is transverse, the basal cell of the two-celled embryo exceeding the other in size. The plane of division of the basal cell is perpendicular to the first, while the apical cell is divided by an oblique wall.

Divisions from this time on appear to be irregular and comparatively slow. By the time the endosperm has completely filled the embryo sac and has resorbed all of the nucellus, the embryo is a club-shaped mass of cells, about ten cells in length, two cells in thickness at the base, and four cells across at the thickest part of the club-shaped expansion (fig. 14). Although the expanded apical end of the embryo contains many more cells than the basal portion, it is not proportionately larger, since the cells of the basal part are a great deal larger than those of the upper part. These basal cells

form a suspensor in the mature embryo. Cell divisions become more frequent at this time, there being apparently no regularity to their plane, since mitoses in various directions may be observed. The only differentiation is found in the suspensor, the cytoplasm in the cells of this structure being more vacuolated than in the other cells of the embryo. Next, the cells of the club-shaped portion become enlarged, thus increasing the size of the embryo. Very soon thereafter the hemispherical end of the embryo becomes flattened across the top (fig. 15). The embryo enlarges still further and the cells in the central region become elongated, forming the primordium of the plerome. The primordia of the cotyledons make their appearance as small protuberances on opposite sides of the flat-topped structure (fig. 16). Embryos of this age which were dissected out and examined showed the cotyledons to be entirely separate and independent structures, there being no indication of a crescentic ridge from which they might arise. They never attain much prominence until germination occurs, and in the ripe seed appear as two small knobs of tissue (fig. 17). There is no indication of a differentiation of cells of either of the growing points of the embryo in the mature seed. The body of the embryo is entirely symmetrical, although the suspensor is slightly curved in nearly all cases.

Historical review

MAGNOLIACEAE

The embryology of *Magnolia virginiana* L., described by MANEVAL (12), corresponds in all essential details to what has been found for *M. grandiflora*. There is built up by irregular cell divisions a pear-shaped mass of tissue, at the base of which is a massive suspensor. The cotyledons then appear simultaneously as two small and independent protuberances at the distal end of the embryo. In the development of the endosperm of this plant, as described by MANEVAL, the first division of the primary endosperm nucleus results in a wall between the daughter nuclei, and both of these nuclei participate in the formation of the endosperm. This is not true for *M. grandiflora*, for while formation occurs very early, there is a brief period when free endosperm nuclei are present.

STRASBURGER (18) published a figure of a very young embryo of *Drimys winteri* Forst. showing a massive suspensor. In the vicinity of the embryo free endosperm nuclei are present. Later stages were not described.

RANUNCULACEAE

OSTERWALDER (15) described the early divisions of the cells of the embryo of *Aconitum napellus* L. as being very irregular, and yet by the time the 28-34-celled stage is reached a dermatogen is differentiated. Such an early differentiation has not been described for any other Ranalean embryo and was not found in either *Magnolia grandiflora* or *Cimicifuga racemosa*. In the further development the cotyledons appear as two small, independent protuberances from the distal end of the embryo, which is attached to the nucellus by means of a short massive suspensor. The endosperm at first consists of free nuclei.

COULTER (7) described the early development of the embryos of *Ranunculus septentrionalis* Poir. and *R. multifidus* Pursh. In both plants the early divisions of the fertilized egg are irregular and a massive suspensor is developed. The later stages were not studied.

MOTTIER (14) studied the embryology of several members of the Ranunculaceae. The young embryos of *Actaea alba* (L.) Mill. and *Delphinium tricornis* Michx., before development of the cotyledons, are described as being typically pear-shaped with a massive suspensor. A crescentic ridge is described as forming at the distal end of the embryo and then bifurcating to form the two cotyledons. MOTTIER has drawn several series of transverse sections through various levels of the embryos to show a deeper cleft between the cotyledons on one side than on the other. That these apparently asymmetrical figures may be an incorrect interpretation due to oblique sectioning of the embryos is shown by figure 18, which is a series of transverse sections through an embryo of *Magnolia grandiflora* which had been carefully examined with a binocular prior to imbedding and found to be perfectly symmetrical, the clefts between the cotyledons being of equal depth. The embryo, however, was sectioned at a slightly oblique angle and the sections correspond to those made by MOTTIER for both *Actaea* and *Delphinium*. Further-

more, this interpretation (that is, a crescentic ridge from which the cotyledons arise) is not comparable with what has been observed in *Cimicifuga racemosa*, for in this plant the cotyledons make their appearance as two entirely independent structures.

In *Aquilegia canadensis* L. the anomalous cotyledonary primordium is said to exist only as a trace, since it bifurcates soon after being laid down, while in *Anemonella thalictroides* (L.) Spach. no such crescentic ridge was observed, the two cotyledons being independent at the beginning.

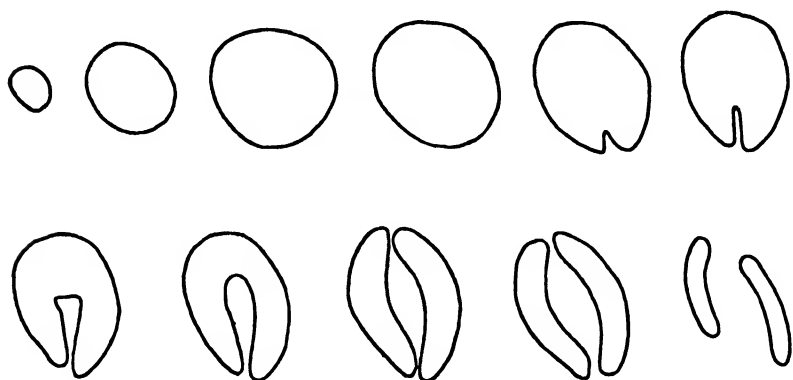


FIG. 18.—Series of transections through mature embryo of *Magnolia grandiflora*

The morphology of the embryo and seedling of *Ranunculus ficaria* L., which is peculiar in having only one cotyledon, has been the source of numerous investigations. WOLTER (19) described the early cell divisions in the formation of the embryo as being very irregular, and at the time the fruit fell there was no plerome, periblem, or dermatogen differentiated. METCALFE (13), in an account of the seedling anatomy of *R. ficaria*, published several figures of the embryo in various stages of development after germination of the seed. These show a suspensor, the single cotyledon, that is at least two cells in thickness all the way to the base, a small "hump" of tissue opposite the cotyledon occupying a position that would normally be the position of the second cotyledon, and between these two structures the apical growing point. METCALFE inter-

preted the small hump of tissue as an aborted cotyledon, while SARGANT (16) regarded the large cotyledon as the result of the fusion of two cotyledons. METCALFE's view is accepted here.

In all cases described for the Ranunculaceae, free endosperm nuclei are formed in the early stages.

BERBERIDACEAE

LEWIS (10) states that in *Podophyllum peltatum* L., *Caulophyllum thalictroides* Michx., and *Jeffersonia diphylla* (L.) Pers. a crescentic ridge forms at the distal end of the embryo and then bifurcates to form the two cotyledons. BUTTERS (1), however, with the use of models and drawings, has shown that the cotyledons in *Caulophyllum* do not arise so but appear as two moundlike outgrowths occupying opposite sides of the margin of the distal end of the embryo. And CLARK (2), with the use of models, drawings, and photographs of actual embryos, has shown that the two cotyledons in *Podophyllum* have their origin as two independent protuberances at the distal end of the embryo. By intercalary growth a cotyledonary tube is developed. In the light of these more recent investigations it seems desirable that further studies be made on the embryology of *Jeffersonia* to test the validity of the assertion that the cotyledons arise as described by LEWIS.

BUTTERS and CLARK agree with LEWIS that in the early stages the embryos in both *Caulophyllum* and *Podophyllum* are entirely undifferentiated bodies, with rather large, massive suspensors. In both plants the endosperm consists at first of free nuclei.

NYMPHAEACEAE

COOK (6) has pointed out that embryo development in this family is subject to considerable variation, and that variation may occur even within a species, unless there has been confusion in the identification of closely related forms. In any event it is evident that there are certain features common to the group as a whole. LYON (11) and YORK (20) have shown that a suspensor is absent in *Nelumbo lutea* (Willd.) Pers. and that the two cotyledonary lobes arise from a common primordium, thus suggesting the presence of a single,

bilobed cotyledon, which is interpreted as such by these workers. COOK (4) described the embryos of *Nymphaea advena* Ait. [*Nymphozanthus advena* (Ait.) Fernald] and *Castalia odorata* (Dryand.) Woodv. & Wood. [*Nymphaea odorata* Dryand.] as being similar to *Nelumbo lutea* in development, except that in *Nymphozanthus* a "latent" suspensor is developed. CONARD (3), however, has shown that a filamentous suspensor may be present in *Nymphaea odorata*, as well as in *N. lotus* (L.) Willd. In a later paper COOK (6) also reported the presence of a filamentous suspensor in *Nymphaea ampla* (Salisb.) DC. and *N. pubescens* Willd., and that such a suspensor may or may not be present in *N. odorata*. He confirmed his previous work as to the presence of a cotyledonary ridge in *Nymphaea odorata* and *Nymphozanthus advena*, as well as that of LYON and YORK as to the presence of the same structure in *Nelumbo lutea*.

It has been shown by COOK (4) that in *Nymphaea odorata* and *Nymphozanthus advena* a wall is formed across the embryo sac following the first division of the primary endosperm nucleus, thus producing a cellular endosperm from the beginning. YORK (20) has noted the same thing in *Nelumbo lutea*. In *Cabomba piauihensis* and *Brasenia purpurca*, however, a few free endosperm nuclei are at first formed (5).

CERATOPHYLLACEAE

Development of the embryo of *Ceratophyllum submersum* L., described by STRASBURGER (17), is strikingly similar to that of *Nelumbo lutea*. A suspensor is lacking and the cotyledonary lobes arise from a common primordium, although there is not so great a difference in the depth of the clefts between the lobes as in *Nelumbo*. A wall divides the embryo sac following the first division of the endosperm nucleus, thus producing a cellular endosperm from the beginning.

Discussion

The manner of development of the various members of the Ranales that have been described is remarkably similar in certain respects and very different in others. The early undifferentiated mass of cells is a character that seems common to all Ranalean

embryos and very closely resembles the condition found in many monocotyledons. Furthermore, Ranalean embryos seem to conform to a general type in having very irregular cell divisions in the early stages and very late differentiation of tissues.

In all cases described for the Magnoliaceae, Berberidaceae, and Ranunculaceae, a suspensor is formed early in the development of the embryo. Although MOTTIER has described the presence of a crescentic ridge of tissue from which the cotyledons arise in *Actaea alba* and *Delphinium tricornis*, this has not been found in other members of the Ranunculaceae, and there is evidence for doubt as to its presence in these two plants. Such a structure is not present in the members of the Magnoliaceae and Berberidaceae that have been investigated, the cotyledons arising as independent structures. In general, then, it may be said that the embryo development, as well as that of the endosperm, in the Magnoliaceae, Berberidaceae, and Ranunculaceae is very similar, but is different from that of the Nymphaeaceae and Ceratophyllaceae. The embryo and endosperm development of *Magnolia grandiflora* and *Cimicifuga racemosa* further strengthens this view.

While there is considerable variation within the Nymphaeaceae with respect to the presence or absence of a suspensor, in other respects the embryology of the group is very uniform and is also very similar to the embryology of the Ceratophyllaceae. The most striking similarity in the embryo development of these two families is the manner of origin of the cotyledonary lobes (from a common primordium), which has been interpreted by some workers (LYON, YORK, COOK) as being a single bilobed cotyledon, and by others (STRASBURGER, CONARD) as being two cotyledons.

It is of particular interest that the endosperm of some of the plants of these two families is cellular from the beginning and resembles the endosperm development of the Aroids in this respect.

Whether the similarities that exist between the embryology of the Nymphaeaceae and Ceratophyllaceae, and the dissimilarities that exist between these two families and the Magnoliaceae, Ranunculaceae, and Berberidaceae, are sufficient justification for suggesting that the Nymphaeaceae and Ceratophyllaceae be placed in the Monocotyledonae, as has been done by a number of workers for the

Nymphaeaceae, is not within the scope of this paper, but they do seem highly significant.

Summary

1. The anatropous ovule of *Magnolia grandiflora* develops in a manner similar to that described by GRAY for *M. tripetala*.

2. The embryo sac of *M. grandiflora* is long and slender, and terminates in a club-shaped expansion at the micropylar end. It contains the usual number of eight nuclei.

3. The endosperm of *M. grandiflora* is cellular very early in its development and finally comes to be a massive structure, containing oil but no starch.

4. The embryo of *M. grandiflora* at first is a mass of undifferentiated cells with a massive suspensor. It becomes pear-shaped by irregular cell divisions. The cotyledons arise from the distal end as independent structures.

5. The development of the anatropous ovule of *Cimicifuga racemosa* shows no marked peculiarity.

6. The embryo sac of *C. racemosa* is of the usual type, having eight nuclei. It is rhombic in shape as seen in longitudinal section.

7. The endosperm of *C. racemosa* in its early stages consists of free nuclei lining the wall of the embryo sac. Wall formation occurs about these free nuclei and the endosperm finally fills the embryo sac. It contains oil but no starch.

8. The embryo of *C. racemosa* is similar to that of *M. grandiflora* in its development, consisting at first of a globular mass of undifferentiated cells. The cotyledons arise from the distal end as independent structures. There is a massive suspensor at the base of the embryo.

9. Development of the embryos of *M. grandiflora* and *Cimicifuga racemosa* corresponds to what has been found in other species of the Magnoliaceae, Ranunculaceae, and also of the Berberidaceae.

10. The embryology of the Ranales as a whole seems to conform to a general type in the early stages, closely resembling the condition found in many monocotyledons; in its later stages it is thoroughly dicotyledonous in the Magnoliaceae, Berberidaceae, and Ranunculaceae.

11. There seems to be some basis for the suggestion that the Nymphaeaceae and Ceratophyllaceae be placed in the Monocotyledonae.

The writer wishes to thank Professor F. K. BUTTERS, of the University of Minnesota, who suggested the problem and gave much valuable counsel during the course of the investigation.

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RELATION OF SOIL TEMPERATURE AND NUTRITION TO THE RESISTANCE OF TOBACCO TO THIELAVIA BASICOLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 494

FRANCES LOUISE JEWETT

(WITH THIRTY-SEVEN FIGURES)

Introduction

Of the diseases which have been studied from the standpoint of the effect of environment on resistance and susceptibility, black root rot of tobacco caused by the fungus *Thielavia basicola* Zopf has received much attention. Field and greenhouse experiments have shown that soil reaction and soil temperature are the most important external conditions determining the severity of the disease. The field experiments of BRIGGS (7), in which he studied the amount of acidity as measured by the lime requirement method, and the later work of ANDERSON (1), DORAN (12), and others in which they measured the intensity of the acidity by determining the pH of the soil, have shown the importance of soil reaction. ANDERSON reports, however, that the range of pH within which tobacco does not become infected with *Thielavia* may vary slightly with the temperature. JOHNSON and HARTMAN (15) tested the effect of various factors, including soil reaction, and concluded that soil temperature is the most important single controlling factor in determining the severity of black root rot of tobacco. Low temperatures, from 17° to 23° C., are the most favorable for development of the disease, while at 29°–30° C. there is very little infection. At 32° C. there is practically no infection, even in the most susceptible varieties of tobacco.

The reasons for this insusceptibility of susceptible varieties at high temperatures are not clear. CONANT (10) reported a distinct correlation between the resistance of the plant and its ability to form cork in advance of the fungus and beneath lesions. The most resistant varieties formed cork readily at low temperatures (20° C.);

the very susceptible varieties formed no cork at low temperatures, but formed it abundantly at the high (30° C.). CONANT states that resistance to *Thielavia* can be accurately measured by the rapidity with which the plant can form cork beneath a lesion.

The first purpose of the present study was to determine the effect of changes in nitrogen nutrition, in conjunction with changes in soil temperature, on the relative resistance of five varieties of tobacco to *Thielavia basicola*. The second purpose was to examine microscopically the roots of these varieties to see the effects of these changes in nutrition and temperature on cork formation. It was also hoped that it could be determined whether a corky layer such as described by CONANT is laid down in advance of or subsequent to fungal invasion, after the initial infection.

Materials and methods

The varieties of tobacco used were: Ordinary White Burley, susceptible; Resistant White Burley, resistant; Connecticut Havana 38, resistant; Havana 142, very resistant; and Xanthia, extremely resistant.

Two cultures of *Thielavia* from the University of Wisconsin, an Italian strain and a Wisconsin strain, proved ineffective in producing infection in tobacco under the conditions of the experiment. New cultures were obtained by isolation from plants grown in infected soil brought from the experimental farm of the University of Wisconsin at Madison. These cultures were used successfully to obtain infection.

The experiments were carried out in the greenhouses of the University of Chicago, using the soil-nutrient temperature tank as described by LINK (19). The plants were grown from seed in sterile soil; when they had reached the six or seven leaf stage, two plants were transferred to each pot of the temperature tank, in which sterile quartz sand was used instead of soil. The temperatures used in all but one series were 28° – 30° C. for one set of tanks and 18° – 20° C. for the other. In one series, grown during extremely hot summer weather, 24° – 26° C. was the lowest soil temperature attainable in the tank.

For each temperature, half of the tanks were given nutrient solu-

tion containing nitrate nitrogen, and the other half, solution lacking the nitrogen. The composition of the nutrient solution given in partial volume molecular concentration is as follows:

SOLUTION	CA(NO ₃) ₂	KH ₂ PO ₄	MgSO ₄	CaCl ₂
Plus nitrate.....	0.0090	0.0045	0.0045
Minus nitrate.....	0.0045	0.0045	0.0090

The pH of the solutions made with distilled water is approximately 5.7 and no adjustment was made. Five hundred cc. of the solution was given to each pot of +N plants three times a week, while the -N plants received 500 cc. twice a week.

Five series of plants were grown. In series I and II the plants were supplied with nutrient solution a month before inoculating with pure cultures of *Thielavia*. By this time the external characteristics of the +N and -N plants were evident, and a nitrate test of the -N plants showed an absence of nitrate nitrogen in the tissues. In these series the pH of the sand was kept between pH 5.6 and 5.9 by watering with acidified tap water. Within this range is the zone in which environmental factors other than pH are limiting, according to ANDERSON and others (1). Plants of series III-V were given nutrient solutions and inoculated from two to three days after placing in the tanks. These series were watered with non-acidified water at regular intervals.

The inoculum for series I-III and V was prepared by growing the fungus in Erlenmeyer flasks on potato dextrose agar. After five to twelve days' incubation, when examination showed an abundance of endoconidia, suspensions of the spores were made by pouring sterile tap water into the flasks, shaking vigorously, and scraping the mat with a sterile inoculating needle. Inoculation was accomplished by clearing away the sand around the plant and pouring some of the suspension directly on the roots. The rest of the suspension was thoroughly mixed with the surrounding sand. In the case of series IV a small amount of infected soil from the field was used as inoculum. This was thoroughly mixed with the sand and was very effective in producing infection.

A set of plants of each temperature and plane of nutrition was kept uninoculated as controls. During the growing period the roots

of the controls of series II and V were wounded by pricking with a fine needle, in order to examine the type of wound reaction shown under the different conditions in the absence of the fungus. In the case of the $-N$ plants, the roots are so small that the wound was made at the base of the stem, or crown, which is below the surface of the soil. Since this region is also attacked by the fungus, it is the region studied in most of the $-N$ plants.

At the end of six weeks the plants were removed from the tanks and measured for total height and relative growth of the roots and tops. The roots were carefully washed and examined microscopically for evidences of infection with *Thielavia*, the chief evidence being the presence of the characteristic chlamydospores.

Material from the control and infected plants was killed and fixed in formal acetic-alcohol, imbedded in paraffin, sectioned on the rotary microtome at $10-12\ \mu$, and stained with Flemming's triple stain.

Results

GREENHOUSE EXPERIMENTS

Table 1 summarizes the results obtained in the tank experiments in the greenhouse. Since no record, except failure to obtain infection, was kept of series III, this series was not included in the table. This summary indicates that between the $+N$ and $-N$ plants there is no difference in resistance and susceptibility which is either sufficiently striking or sufficiently consistent to be evident from a limited number of small scale experiments.

Between the high temperature and the low there is a difference both in the number of varieties affected and in the severity of the infection. At the low temperature the number of varieties showing infection is greater, since at this temperature the more resistant varieties immune at high temperatures become infected. The infection is also more severe at the lower temperature, as can be seen from the greater degree of decay of the roots and the greater stunting of the plant as a whole.

Although the number of varieties affected increases with a decrease in temperature, the order of severity of infection parallels the order in which the five varieties used are rated as to suscepti-

bility. This is true for all temperatures and for both +N and -N plants. The combined effect of temperature and the genetic constitution of the variety is seen in series I, in which the fungus culture was very weakly aggressive. The only undoubted case of infection occurred in the most susceptible variety at the low temperature.

The results of these experiments are in agreement with the general finding of others, that of the environmental factors tested, soil temperature is the most important one influencing the expression of

TABLE 1

COMPARATIVE INFECTIONS OF FIVE VARIETIES OF TOBACCO UNDER +N AND -N CONDITIONS AT DIFFERENT TEMPERATURES (IN DEGREES C.)

VARIETY	SERIES I				SERIES II				SERIES IV				SERIES V			
	+N		-N		+N		-N		+N		-N		+N		-N	
	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18-20	28- 30	18-20	28- 30
Ordinary White Burley	+	-	+	-	-	-	-	-	+	+	+	-	++++	++++	++++	++++
Resistant White Burley	-	-	-	-	-	-	-	-	+	+	+	-	++++	++++	++++	++++
Havana 38	-	-	-	-	-	-	-	-	+	±	+	-	++++	++++	++++	++++
Havana 142	-	-	-	-	-	-	-	-	±	±	±	-	++	++	++	++
Xanthia	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	±

- = no infection; ± = slight infection; +, ++ = fairly heavy to heavy infection; +++ = severe infection.

resistance and susceptibility of varieties of tobacco to *Thielavia*. The results also show the importance of the breeding of resistant strains as a practical method of control, since even at the low temperatures most favorable for the disease, resistant varieties are not so severely affected as susceptible varieties, although they may not be completely immune.

MICROSCOPIC EXAMINATION OF ROOTS

UNWOUNDED CONTROLS.—At both the high temperature (28°-30° C.) and the low (18°-20° C.), a somewhat more regular and compact periderm was found in the +N control plants than in the -N ones. At both temperatures +N susceptible Ordinary White Burley

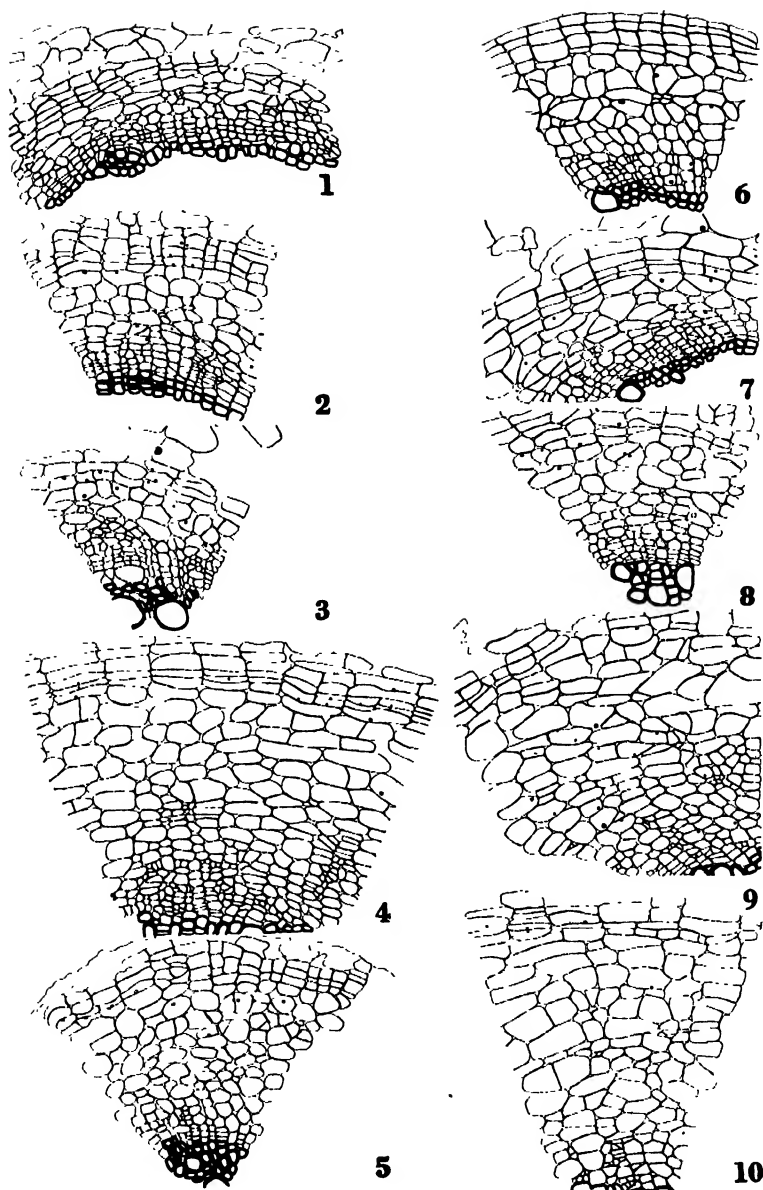
showed as well developed periderm as very resistant Xanthia. In all cases, however, the White Burley was much more severely infected than Xanthia, which remained almost entirely free of infection. The same was true of Resistant White Burley, which is more susceptible than Xanthia. At both temperatures and planes of nutrition the most extensive and continuous periderm was found in the roots of Havana 142, a very resistant variety although not so resistant as Xanthia.

At both temperatures very resistant Xanthia showed more regular periderm formation in the $-N$ plants than did susceptible White Burley.

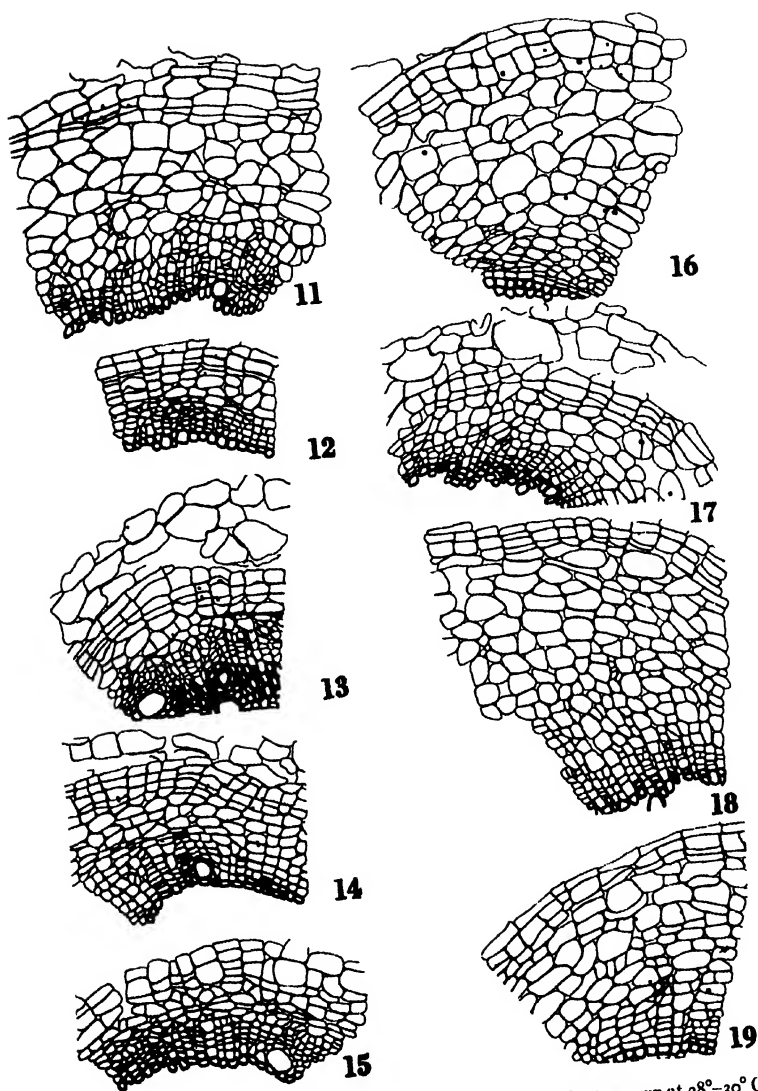
Havana 38, which is intermediate as to resistance, is the only one which showed more regular periderm formation at higher temperatures than at low. This was true for both $+N$ and $-N$ plants. Comparing Havana 38 with Xanthia, it was found that at the higher temperature $+N$ Havana 38 showed slightly better developed periderm than $+N$ Xanthia, while $-N$ Havana 38 had a periderm which was at least as well developed as that of Xanthia. At the lower temperature Xanthia formed a more extensive periderm in both $+N$ and $-N$ plants. At both temperatures Havana 38 was much more severely affected than Xanthia.

Figures 1-19 show portions of the roots of the different varieties studied, giving in each case the region of the best developed periderm noted. The development of a protective layer was by no means uniform, either in extent around the root or in depth and compactness of the periderm cells. Havana 142 showed the most regular development. The thickness of the walls of these cells and their reaction to stains indicated that they were not always very heavily suberized. Their efficiency as a factor in resistance is doubtful.

The base of the stem which is underground, and which may become as heavily infected as the roots, showed no regular protective periderm layer. The epidermal cells were rather heavily suberized, as were the subepidermal cells in some cases. In certain regions a subepidermal periderm had developed, although in many stems no very extensive periderm was formed at any point, nor was the periderm uniform around the whole stem. One side of the stem often



FIGS. 1-10.—Portions of cross sections of roots of control plants grown at 18°-20° C., showing comparative peripheral periderm development. Figs. 1-5, +N: 1, Xanthia; 2, Havana 142; 3, Havana 38; 4, Resistant White Burley; 5, Ordinary White Burley. Figs. 6-10, -N: 6, Xanthia; 7, Havana 142; 8, Havana 38; 9, Resistant White Burley; 10, Ordinary White Purley.



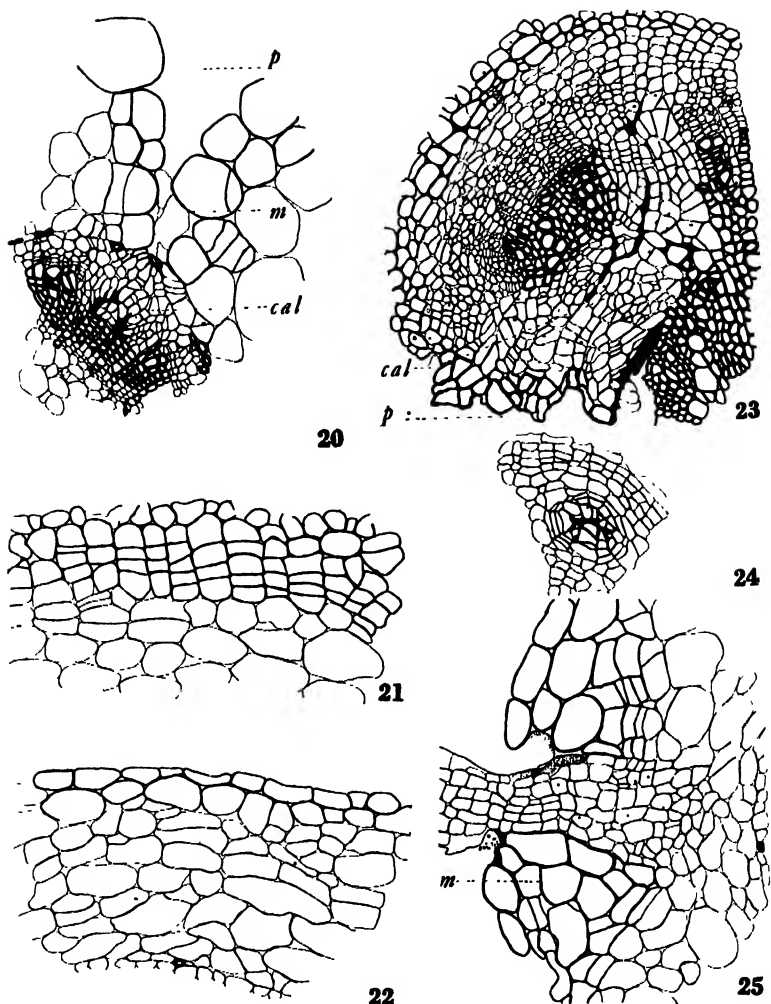
FIGS. 11-19.—Portions of cross sections of roots of control plants grown at 28°-30° C., showing comparative peripheral periderm development. Figs. 11-15, +N: 11, *Xanthia*; 12, Havana 142; 13, Havana 38; 14, Resistant White Burley; 15, Ordinary White Burley. Figs. 16-19, -N: 16, *Xanthia*; 17, Havana 38; 18, Resistant White Burley; 19, Ordinary White Burley.

showed a periderm of several cells in thickness, while the other side had only the suberized epidermal cells as protection (figs. 21, 22). So far as could be seen, the structure of the stem with respect to periderm formation was unchanged in relation to differences in temperature and nutrition. No marked differences were observed in the several varieties of tobacco used.

PROTECTION AT BASE OF BRANCH ROOTS.—Examination of the bases of branch roots emerging from both stems and roots showed no consistent formation of periderm as a protective layer in the areas exposed by the breaking through of the emerging root. In the case of secondary roots there was only the slightest evidence of the continuous peripheral cork layer described by CONANT (10). There were very definite reactions in the cortex of the stem in response to the injury made by the emerging adventitious root (fig. 25). The staining reaction indicated that the composition of the cells of the walls of the stem adjacent to the emerging root had changed to a substance staining like lignin or suberin. The walls of such cells also appeared somewhat thickened. Near the periphery of the stem there was some cell enlargement accompanying the change in nature of the cell wall. Very often cell divisions resulted in the formation of a weak periderm-like layer delimiting the wound made by the root emergence. Gum deposits were seen in the small pockets made by the destruction of the cells of the stem. There was apparently no correlation between the intensity of the reaction and the relative resistance of any variety examined. The reactions described were entirely comparable to the responses to other wounds in the cortex, a condition noted by BOYLE (5) in relation to emerging secondary roots in flax.

Examination of cross sections of control roots of as nearly comparable stages of development as possible showed that the amount of normal periderm formed by the five varieties of tobacco under the different conditions of the experiment could not be clearly correlated with the order of the rating of these varieties as to their susceptibility and non-susceptibility to *Thielavia*.

REACTIONS TO INJURY.—The most common response to injury was the change in constitution of cell walls in mechanically injured and infected areas. This change is evident from the reaction of the



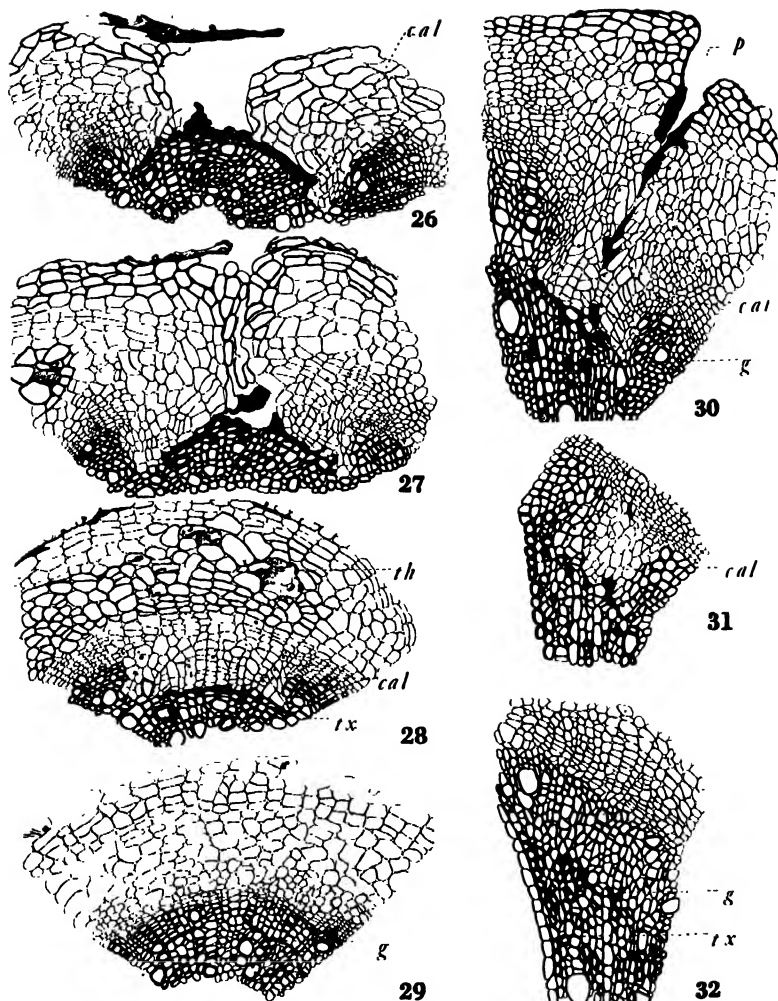
FIGS. 20-25.-Fig. 20, needle wound and reactions in stem, showing metacutinization of cortical cell walls, wound gum, cell divisions in cortex, and callus formation (*p*, path of needle; *m*, metacutinization; *cal*, callus); Havana 142, 18°-20° C., -N; control. Figs. 21, 22, portions of periphery of same cross section of stem base, fig. 21 showing subepidermal periderm on one side and fig. 22 lack of periderm on other side of stem; Havana 142, 18°-20° C., +N; control. Fig. 23, isolation of portions of xylem by callus development stimulated by injury with needle; cells bordering path of needle show metacutinization of walls, indicated by heavier lines; Ordinary White Burley, 18°-20° C., +N; control. Fig. 24, small wound in cortical region of root, showing deposit of gum surrounded by cells reacted by dividing; Ordinary White Burley, 18°-20° C., +N; control. Fig. 25, wound reactions around emerging adventitious root and showing evidence of cell divisions and wound gum formation; Havana 38, 24°-26° C., -N; control.

walls to stains. Cells whose walls stained orange in healthy roots, indicating cellulose with Flemming's triple stain, change to the red of lignified or suberized walls in injured regions. This change in constitution is comparable to the metacutinization of cell walls in injured areas described by KÜSTER (18). In control plants, cells bordering wounds of even very small extent show this reaction. In infected plants this response is found in cells adjacent to those containing the fungus as well as in the infected ones themselves.

Metacutinization is often accompanied by thickening of the wall of infected cells or of cells nearest the region destroyed by the fungus or by mechanical injury. Marked thickening of xylem elements is a characteristic response to injury (figs. 26-28, 32).

In addition to these changes in cell walls, deposits of a dark staining substance, apparently wound gum, are found in injured areas. Small wounds in the cortex result in the formation of pockets of gum surrounded by cells which have reacted by changes in the cell wall and by cell divisions (fig. 24). Similar pockets are found in injuries at the periphery of the root. Heavy gum deposits are seen in severely infected regions and in deep mechanical wounds. These deposits border the area of destroyed cells of the cortex and phloem, and are found on the face of the xylem where the cambium and some of the xylem elements have been destroyed (figs. 26, 27, 30). Gum in and between the xylem elements is one of the first wound reactions visible at a distance above or below the area actually destroyed (figs. 29, 32). In less severe mechanical injury and in infection this substance is seen as a coarsely granular material. In diseased roots it is often seen in the cells containing the fungus, where it appears as a granular coating on the hyphae (fig. 36).

Stimulation to cell division is a further reaction to injury. More or less regular divisions occur in the affected cells around some wounds (figs. 24, 33), but for the most part the divisions are irregular and do not result in the formation of a layer anatomically comparable to a compactly organized periderm. In deep lesions of both mechanical and fungal origin living cells bordering the destroyed areas are stimulated to callus formation (figs. 26-28, 30, 31). Involved in this formation are the cells of the cambium undestroyed at the edges of the wound and the parenchyma cells of the other



FIGS. 26-32.—Comparison of fungus injury with mechanically induced wound: Figs. 26-29, injury caused by *Thielavia basicola*; Havana 142, 18°-20° C., -N; fig. 26, region of greatest tissue destruction; wound gum on face of xylem and callus developing at edges (*cal*, callus). Chlamydospores of *Thielavia* in debris at periphery of root; fig. 27, region just beyond that shown in fig. 26; fig. 28, beyond fig. 27, approaching edge of region which had been destroyed (*th*, hyphae of *Thielavia*); metacutinization of cortical cell walls indicated by heavy lines; wound response in xylem seen in thickening of some xylem elements (*tx*); fig. 29, beyond region of tissue destruction; evidence of injury seen in wound gum (*g*) in xylem. Figs. 30-32, wound mechanically induced by pricking; Ordinary White Burley, 28°-30° C., +N; fig. 30, just above center of path of needle (*p*); fig. 31, region beyond that of fig. 30, approaching limits of the injury, but responses seen in callus and wound gum formation and thickening of xylem elements; fig. 32, beyond region of cell destruction; evidences of wound reaction in xylem in gum deposits and thickened xylem elements.

tissues bordering the injured region. Stimulation of the xylem parenchyma to division often results in the irregular development of the xylem due to the formation of areas of callus tissue isolating one part from the rest (fig. 23). This irregular development was seen in both types of injury, and was also mentioned by CONANT as one of the reactions to the invasion of *Thielavia*.

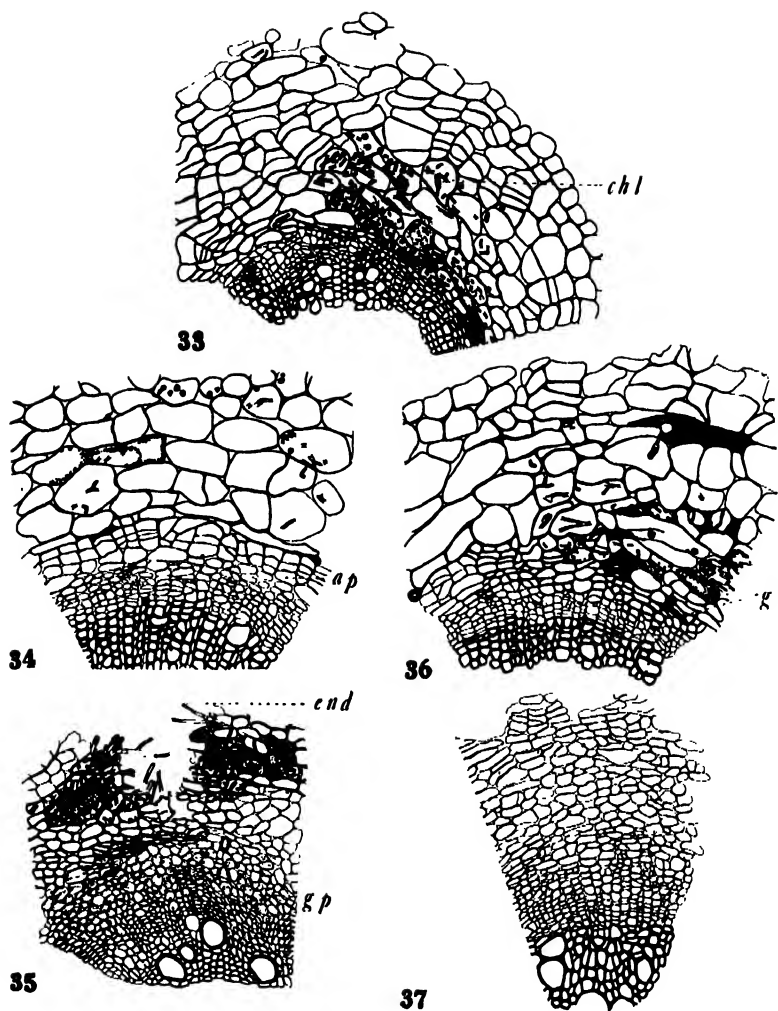
The callus tissue effectively heals the wound. This type of reaction to local injury was found in stems as well as in roots in the five varieties studied under the different conditions of the experiment (fig. 20). No differences were noted in amount or type of tissue formed which might be correlated with resistance and susceptibility.

One reaction seen in infected roots was not seen in those mechanically injured. Beneath heavily infected areas, the protoplasm was more granular and stained much more deeply than the protoplasm in similar cells in healthy areas of the same root (figs. 35, 37). This response to the fungus was found in these roots only when infection was rather severe. TISDALE (26) described a similar appearance in the cells of infected flax roots, and suggested that the change might be due to a chemical reaction brought about by the presence of the fungus.

These microscopic examinations of roots and crowns of tobacco plants infected with *Thielavia basicola* under the conditions of this experiment failed to corroborate the findings of CONANT with respect to periderm formation around lesions resulting from the presence of the fungus. A comparison of injuries made by *Thielavia* with those induced mechanically showed that the reactions to wounding were of the same type regardless of the cause of injury, and that other reactions were much more frequent than periderm formation.

Discussion

Although series I-III were of no great value in providing answers to the main questions under investigation, they were of interest since they apparently showed that *Thielavia basicola* may lose its virulence after long culturing on artificial media. This is not in accordance with the experience of JOHNSON and HARTMAN (15), who found no evidence in the literature or in their experiments of variation in virulence due to the age of the culture or to differences in



FIGS. 33-37.—Fig. 33, infected stem base showing cell divisions between fungus and periphery of stem; infected cells and those adjacent show metacutinization of walls (*chl*, chlamydospores of *Thielavia*); Havana 38, 28°–30° C., –N. Fig. 34, evidences of activity in pericycle adjacent to infected cells of cortex; walls of cortical cells and outer cells of pericycle, metacutinized (*ap*, activity in pericycle); Ordinary White Burley, 18°–20° C., +N. Fig. 35, infected root showing granulation of protoplasm in cells beneath heavily infected area (*end*, endoconidium; *gp*, granular protoplasm). No evidence of periderm formation around fungus lesion; Ordinary White Burley, 28°–30° C., +N. Fig. 36, same stem base shown in fig. 34. No evidence that activity in pericycle has formed barrier of periderm cells in advance of fungus. Walls of infected and adjacent cells metacutinized, and granular gumlike material seen in some cells. Fig. 37, healthy portion of root shown in fig. 35; protoplasm of cells not granular.

strain, and that *Thielavia* is apparently stable in its pathogenicity. PETERS (21) and SATTLER (23) on the other hand both report loss of virulence after long cultivation on artificial media, two to three years according to PETERS. Both also report evidences of different biologic races of *Thielavia*.

The relation of soil temperature to disease has been fully discussed by JONES, JOHNSON, and DICKSON (16) and others. The effects of temperature on the resistance and susceptibility of tobacco to *Thielavia* have been well established by field observations and experiments, although the mechanism by which high temperature increases resistance is not understood.

Work on the relation of nutrition to resistance and susceptibility shows that, owing to the many factors involved in the nutrition of a plant, it is difficult to make generalized statements. FISCHER and GAÜMANN (13) state that in general, high nitrogen nutrition increases susceptibility, while high phosphorus, potassium, and calcium lessen it. They state that it is necessary to have extremes of high or low nutrition with these elements for the changes in resistance to be evident. Furthermore, the effects of these elements are not always the same. In certain diseases, high nitrogen may increase resistance and high phosphorus decrease it.

SCHAFFNIT and VOLK (24), working on the relation of various individual nutrients to resistance of both herbaceous and woody plants, found that plants with a decided lack of nitrogen were only slightly susceptible while those with a decided excess were especially susceptible. This was true wherever susceptibility and resistance were independent of the age of the host. In plants in which age affects resistance, this relationship to nitrogen may be reversed in the early stages of growth, while in the older stages the +N plants are again more susceptible.

LINK and WILCOX (20) report that succulent shoots of Stayman apple trees, such as formed under high nitrogen conditions, are susceptible to *Erwinia amylovora*, while the -N shoots are only slightly disposed toward the organism.

In the case of stem rust of wheat, a high amount of nitrogen is known to increase development of the rust. JOHNSON and JOHNSON (14) studied the nitrogen content of mature and immature parts of

the wheat plant in relation to resistance to the rust. They found that the mature parts had a higher nitrogen content than the immature parts, and at the same time were more resistant than the immature. They concluded that resistance cannot be due to the actual increase in nitrogen content, as believed by GASNER and FRANKE, but that in the older tissues other factors enter in, such as the increased resistance offered by the more mature cells or the development of some substance having an inhibiting effect on the fungus. They believe that resistance to rust cannot be explained on a directly nutritional basis.

Reports on the effects of nutrition on resistance to root fungi are not numerous, and the conclusions are somewhat divergent. SATTLER (23) found that beans were more susceptible to *Thielavia* under conditions in which nitrogen was either completely lacking or greatly in excess.

COOK (11) found that both resistant and susceptible tomato plants showed a high frequency of infection with *Fusarium lycopersici* under $-N$ conditions, although the symptoms of wilt were infrequent in $-N$ plants. Seedlings of a resistant variety were infected under both $+N$ and $-N$ conditions, showing symptoms similar to those of susceptible varieties.

REED and FRÉMONT (22) found that citrus roots growing in soils which had been without fertilizer for seven years offered little resistance to the invasion of mycorrhizal fungi and were unable to digest intracellular mycelium. Roots in soils having had annual cover crops and application of stable manure offered a definite resistance to the fungus.

THOMAS (25) states that trees infected with *Armillaria mellea* develop an antagonism to the fungus only when the plant is in an active healthy state, a condition which implies at least no lack of nitrogen.

The tobacco plants examined in the present study showed no anatomical modifications in response to the presence of *Thielavia* which could be correlated with resistance. No such periderm layers as described by CONANT were found; and while evidences of cell divisions around some infected areas were noted, these were not found consistently enough, either in a given diseased root or under

a given set of conditions, to be considered a factor in resistance. One of these regions showing such activity in the pericycle occurred in susceptible White Burley at the low temperature, the temperature condition under which the plant was the most susceptible (fig. 34). Other parts of the same crown showed that the fungus had not been stopped by a periderm-like barrier, but had penetrated the phloem almost to the xylem (fig. 36). This same irregularity of cell division in response to the presence of the fungus was seen in the other varieties used in this experiment.

Periderm formation has received much attention as a possible factor in determining the ability of a plant both to resist initial invasion and to prevent further penetration after invasion. The work of ARTSCHWAGER and STARRETT (4) on sweet potato and gladiolus shows the importance of the ability of the root to heal rapidly the wounds made in harvesting. Unhealed wounds are portals of entry for pathogenic organisms during storage. Under conditions of high temperature and relative humidity, when periderm is formed most rapidly, rotting in storage is much decreased. According to APPEL (2) the cork layer must be thick, for many fungi are able to penetrate thin cork layers.

As to the effectiveness of a corky layer in preventing continued penetration after initial invasion, there is a variety of findings. BROOKS (8) states that with certain varieties of plants, resistance to some fungus diseases is related to the ability of the host to form cork barriers readily in response to attempted invasion. This cork may apparently completely check the advance of the fungus, so that its harmful effect on the plant as a whole is decreased. In the case of rough bark of aspen, caused by *Macrophoma tumefaciens*, as described by KAUFERT (17), the fungus penetrates the periderm. The host forms a new periderm in response to the wounding; the fungus penetrates this, and the alternate penetration and formation of new layers continue until there is a very thick layer of cork. This slow progress of the fungus restricts the organism to the outer cortex although it may be present there for years.

THOMAS (25) found that the wound made by the invasion of *Armillaria mellea* in resistant hosts is walled off by a periderm, but

he doubts the significance of the cork layer as a factor in resistance, since the fungus easily breaks through such layers.

Much of the literature indicates that where cork formation is observed, it is not considered to be the only factor operative in resistance but functions in conjunction with other factors. APPEL (2) found in black leg of potato that the rapidity with which the potato is able to form cork in response to the wound is related to the ability to resist further invasion. But he also states that the efficiency of the cork layer is aided by conditions that diminish the growth of the bacteria, giving the wound time to heal before the bacteria can penetrate. In the case of fungus diseases such a newly formed cork is not effective, he thinks, as fungi are able to penetrate it.

LINK and WILCOX (20) found that a periderm walled off lesions made by *Erwinia amylovora* in apple shoots. But they also noticed that there was usually a band of unaffected cells between the bacteria and the periderm. The bacteria were evidently stopped first and the periderm was formed later as a regenerative reaction to the wound, rather than in advance as a defense mechanism.

TISDALE'S work on flax is often quoted as describing a situation in which the flax wilt fungus is prevented from invading the vessels in resistant varieties by the formation of a corky barrier between the fungus and the vessels. TISDALE in fact pointed out that the formation of a corky barrier was but one of a number of cellular changes, and that a substance was formed by the host cells which was possibly harmful to the fungus. He surmised that failure of the fungus to penetrate the periderm layer may be conditioned only after the fungus has been weakened by some activity on the part of the protoplasm of the host. Thus resistance resulted from a combination of the weakening of the parasite and the formation of a corky barrier.

The failure of BOYLE (5) to find in flax roots a corky layer such as described by TISDALE may be additional evidence that periderm formation is not the primary factor in resistance in flax roots.

BRAMBLE (6) was uncertain of the complete effectiveness of a wound periderm in preventing the spread of *Endothia parasitica* in chestnut. He thought, however, that it might delay or stop the ad-

vance of the fungus under conditions where the fungus grows slowly because of some other inhibiting factor. BRAMBLE concluded that wound periderm formation should at least be considered among the factors contributing to resistance to *Endothia*.

The metacutinization and thickening of the cell walls in infected areas might offer some mechanical resistance to invading fungi. But there was no evidence in the tobacco roots of this experiment that the fungus is restricted by these features, nor was there any correlation between their occurrence and resistance. From the comparable reactions in mechanically injured roots it is concluded that these are the usual wound responses.

Both TISDALE and BOYLE described metacutinization in flax, but from microchemical tests BOYLE concluded that the substance deposited in the cell walls is lignin-like. He found that this metacutinization is of the same type as that found in reactions to mechanical injuries, and he thought that the deposition of lignified material on the cell wall could not be considered a primary factor in resistance. BRAMBLE described similar reactions in chestnut, and concluded that lignification of cells was not of great importance in resistance to further invasion, since these cells also become infected.

BOYLE, however, found that a greater stability of the cortical cell walls was correlated with resistance. This stability was not evident anatomically but was detected by testing the resistance of the walls to hydrolysis with sulphuric acid and determining the amount of non-hydrolyzable materials in the walls.

Callus formation occurring as a result of severe injury is primarily a regenerative rather than a directly defensive reaction. Its occurrence in regions infected severely enough to result in the destruction of areas of cells suggests that the fungus has been weakened or stopped in its progress, giving the cells which have not been destroyed the opportunity to divide and begin regeneration. The newly formed callus is apparently not infected by the fungus remaining near the new tissue. This may be a further indication that the aggressivity of the fungus has been decreased, either because the organism has been weakened by some substance formed in the cells in response to the fungus, as suggested by TISDALE, or because the nutritive conditions are no longer favorable to the fungus. On the

other hand, the lack of infection in the new callus may suggest some chemical characteristic of this tissue as yet not understood. Anatomically callus does not offer a mechanical barrier to invading fungi, because of its thin walled cells.

Since no anatomical modifications were found in the tobacco plants of this experiment which could explain resistance and susceptibility to *Thielavia*, it seems probable that the mechanisms of resistance are chemical. TISDALE's suggestion of a fungus-weakening substance, the description by THOMAS of the development in roots of an antagonism toward *Armillaria mellea*, the mention by others of the formation of inhibiting substances, and the work on acquired physiological immunity in plants summarized by CHESTER (9), all show the trend of thought in the direction of a chemical basis for resistance. More specifically, the reports of ARNAUDI (3) that he was able to increase the resistance of tobacco plants to *Thielavia* by treating the plants with vaccines is suggestive of an approach to the understanding of the problem of resistance in the case of black root rot of tobacco.

Summary

1. Plus and minus nitrogen tobacco plants, of the five varieties used in this experiment, showed no differences in resistance and susceptibility to *Thielavia basicola* at any given temperature. Changes in nutrition do not change the order of resistance.

2. These experiments confirm the evidence of other investigators that soil temperature is the most important environmental condition determining the expression of resistance and susceptibility of tobacco to the fungus. The breeding of resistant varieties is the most important practical method of control of black root rot.

3. Periderm formation in control plants at different temperatures and under +N and -N conditions could not be correlated with resistance.

4. No evidence was found of the formation of periderm in advance of the fungus or around lesions made by the fungus.

5. Response to the wounds made by the fungus was similar to the reaction to mechanical wounds.

6. Callus formation in roots and stems in response to injury by the fungus appears to be regenerative rather than defensive.

7. Resistance to *Thielavia* under certain environmental conditions is not determined primarily by anatomical modifications in the root or crown of the plant.

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NITROGEN AND CARBOHYDRATE METABOLISM OF KIDNEY BEAN CUTTINGS AS AFFECTED BY TREATMENT WITH INDOLEACETIC ACID

NEIL W. STUART

(WITH FOUR FIGURES)

Introduction

It is well known that a large group of chemical growth substances are capable of inducing root formation on cuttings of many species, being particularly effective with softwood and herbaceous cuttings. Little is known, however, as to the nature of the chemical changes brought about within the cutting as the result of treatment with any of these growth substances.

STUART and MARTH (5) found that cuttings of *Ilex opaca* treated with indolebutyric acid accumulated more sugars in their stems prior to root emergence than did similar untreated cuttings. COOPER (2) had previously referred to this mobilizing action of growth substances in woody cuttings. He postulated that the action of indoleacetic acid in root formation is primarily the mobilization of naturally occurring root forming substances.

Regarding the effect of growth substances on the chemical changes of intact plants, rather than of cuttings, MITCHELL and MARTIN (4) applied 3 per cent indoleacetic acid-lanolin mixture to the first internode of etiolated kidney bean seedlings, and found that this treatment tended to restrict the withdrawal of materials from the cotyledon and their deposition in the treated portions and parts of the plant above the point of treatment. On the other hand, hypocotyls of treated plants gained more in dry weight following application of the acid than did similar parts of control plants. BORTHWICK, HAMNER, and PARKER (1) reported that in tomato stems treated with indoleacetic acid-lanolin mixture, proteins increased and starch decreased in those areas where cell divisions became most abundant. Their observations were made by means of microchemical tests.

The purpose of the present study was to determine the nature and

magnitude of the changes in dry substance as well as the distribution of nitrogen and carbohydrates in cuttings treated with indoleacetic acid. Cuttings of kidney bean seedlings were found to be very suitable for this study, being uniform and highly reactive to growth substances.

Materials and methods

Five crops of beans (*Phaseolus vulgaris*, Calapproved selection) were grown in the greenhouse during the spring and summer of 1938. The beans were grown in 300 5-inch pots each containing five beans planted at a uniform depth and position in a mixture of equal parts by volume of soil and peat. The beans were watered with tap water and received one application of a complete nutrient solution containing also the known essential minor elements.

Cuttings were made on the ninth day after planting, when the second internode was about 1 cm. in length (fig. 1). The hypocotyls were severed just above the surface of the soil. Except as otherwise noted, the cotyledons and the top of the plant above the heart-shaped leaves were removed when the cuttings were made. The cuttings, carefully selected for uniformity, were treated by standing them in bundles of twenty-five in 100 cc. beakers containing 50 cc. of 0.01 per cent indoleacetic acid or tap water for 4 hours. In preliminary experiments this treatment produced the maximum response. After treatment the cuttings were rinsed in tap water and set in media of washed sand contained in a glass inclosed propagating frame within the greenhouse. The frame was shaded so that the light intensity at the surface of the leaves was from 600 to 1000 foot candles. The temperature in the greenhouse ranged from 70° to 100° F. Each sample, consisting of 100 cuttings, was removed from the sand at 9 A.M., washed, and separated into upper and lower hypocotyls by division at the middle, first internode, petioles, and blades. These fractions were dried for 20 hours at 80° C. in a forced draft oven.

The sugar content of the cuttings was determined as previously described (5). Starch was digested with fresh saliva, followed by acid hydrolysis. Total nitrogen was determined by the Official Gunning method. No nitrates were present in the samples.

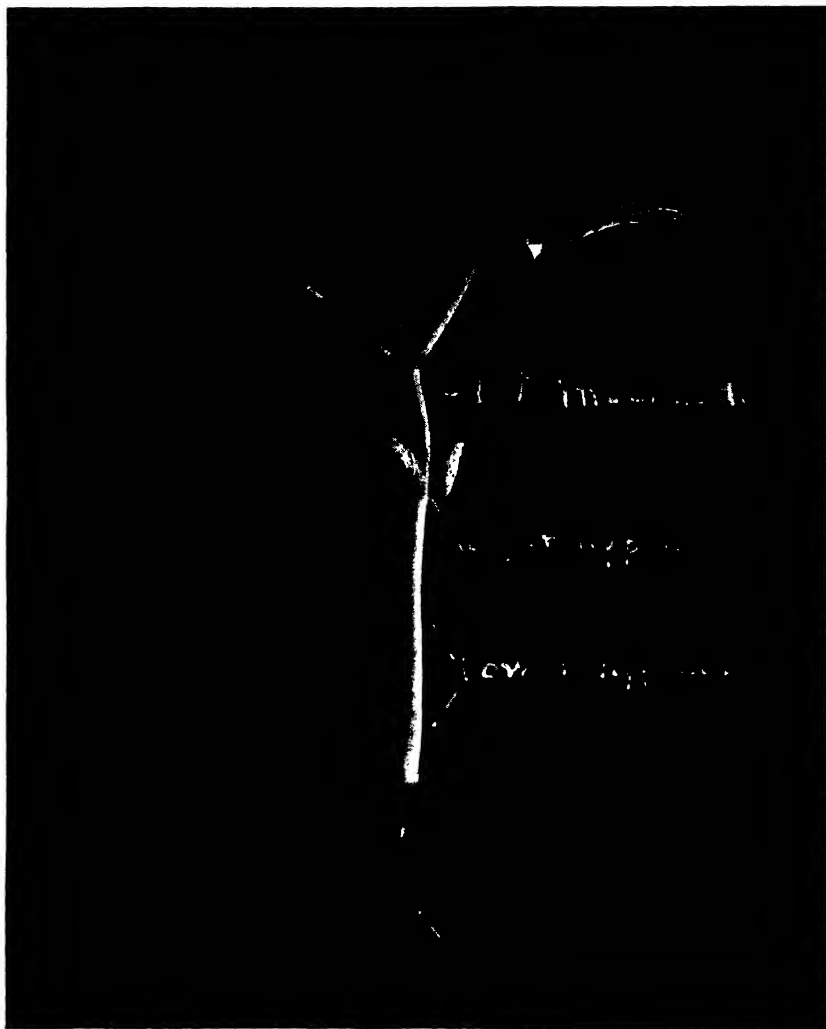


FIG. 1.—Stage of kidney bean seedling when cuttings were made

Experimental results

INFLUENCE OF INDOLEACETIC ACID ON GROWTH OF BEAN CUTTINGS

Untreated cuttings produce a few roots and the base of the hypocotyl enlarges slightly by about 100 hours. During this period the second internode elongates and the first trifoliolate leaves unfold. Similar cuttings treated 4 hours with 0.01 per cent indoleacetic acid produce four dense rows of roots on the hypocotyl, which enlarges greatly, particularly the lower portion. Roots are produced on treated and untreated cuttings at about the same time. Meanwhile the growth of the second internode is suppressed for several days, but gradually is resumed and the trifoliolate leaves are unfolded (fig. 2). The response to treatment depends upon the concentration of the solution as well as upon the time of exposure. The responses obtained when cuttings are treated with 0.01 per cent indoleacetic acid for periods ranging from 1 minute to 4 hours are shown in figures 3 and 4. Lengthening the period of exposure increases the number of roots and size of hypocotyl (fig. 3), while the growth of the second internode is progressively depressed (fig. 4). Exactly the same effect is obtained if cuttings are treated for a uniform length of time with solutions of different concentrations of indoleacetic acid. The cuttings shown in figures 3 and 4 were treated with solutions ranging in concentration from 0.0001 to 0.01 per cent. Quantitative dry weight determinations were made, confirming the conclusions reached with respect to the visible growth responses.

INFLUENCE OF INDOLEACETIC ACID ON DISTRIBUTION OF DRY WEIGHT

The cuttings were grown under conditions of low light intensity and high temperature. As a result, there was little net increase in weight during the period in the propagation frame (tables 1 and 2). The untreated cuttings increased slightly more in dry weight than the treated ones.

There were, however, striking changes in the distribution of materials within the cuttings. The treated hypocotyls in a period of 120 hours nearly doubled in dry weight while the untreated hypocotyls increased only 20 per cent. On the other hand, the first inter-

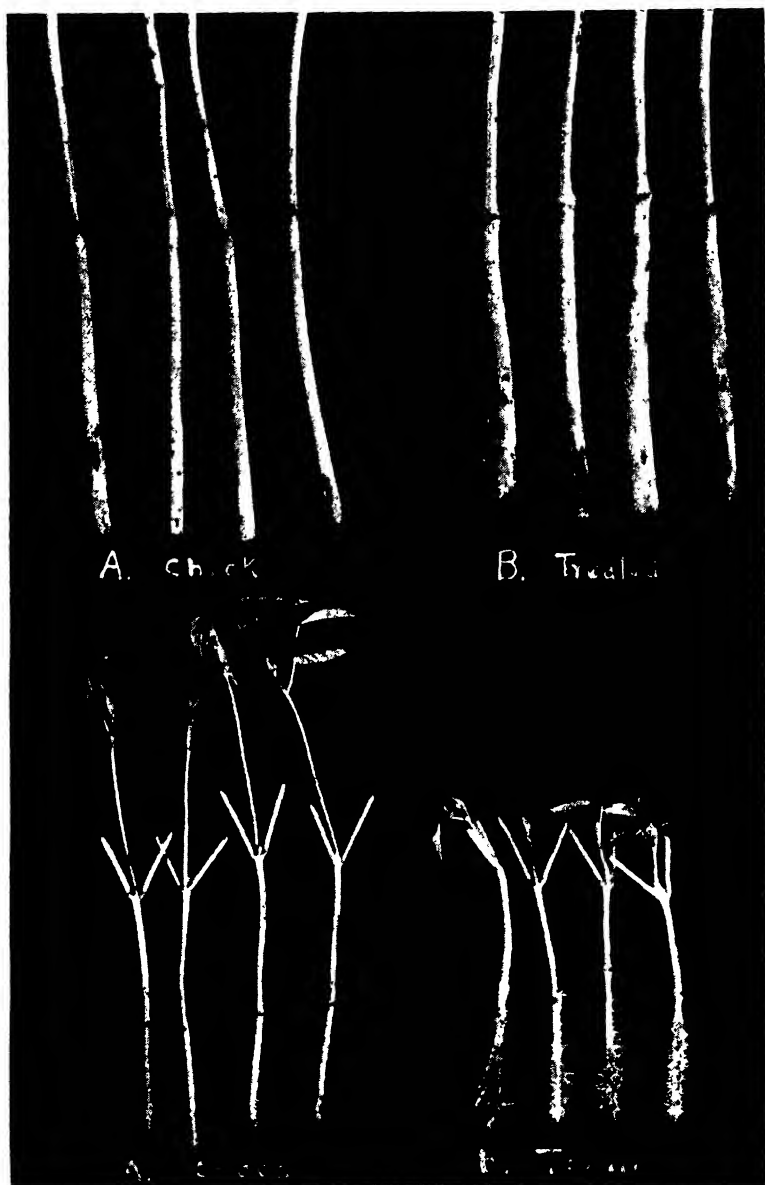


FIG. 2.—Upper: *A*, control cuttings 96 hours after making them; *B*, treated 4 hours with 0.01 per cent indoleacetic acid, 96 hours after making cuttings. Note increase in size of hypocotyls. Lower: same as upper, showing whole cuttings (except primary leaves) 120 hours after making them.

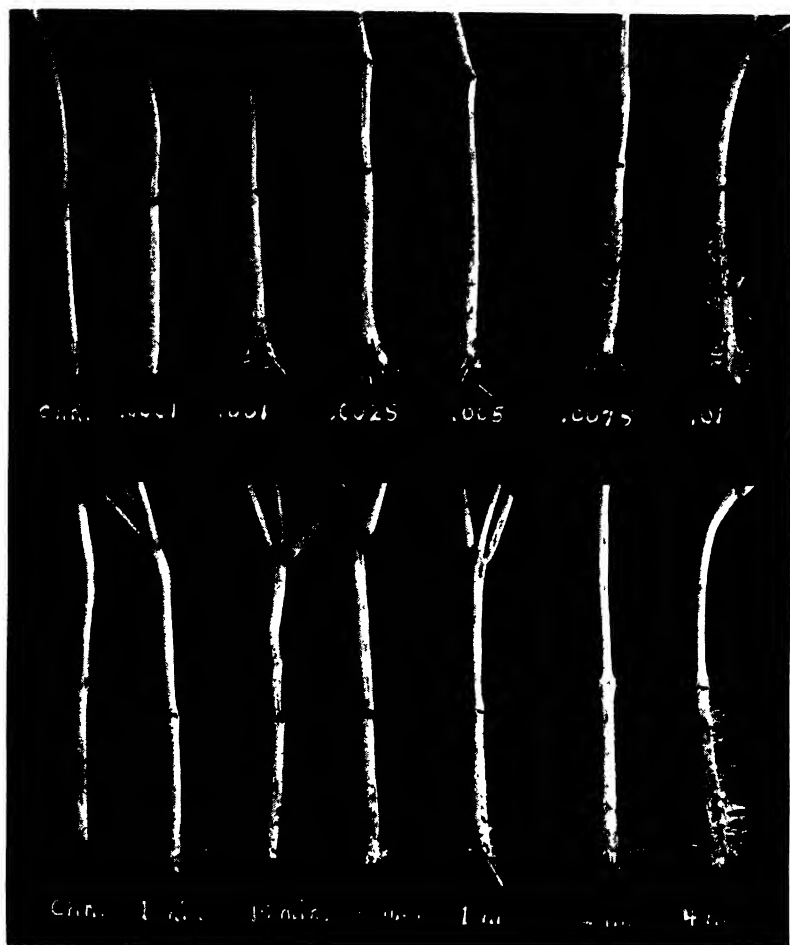


FIG. 3.—Upper: response of cuttings treated 4 hours with increasing concentrations of indoleacetic acid. Left to right, 116 hours after treatment. Note progressive increase in number of roots and size of hypocotyl. Lower: response when treated with 0.01 per cent indoleacetic acid for increasing lengths of time, 116 hours after treatment.

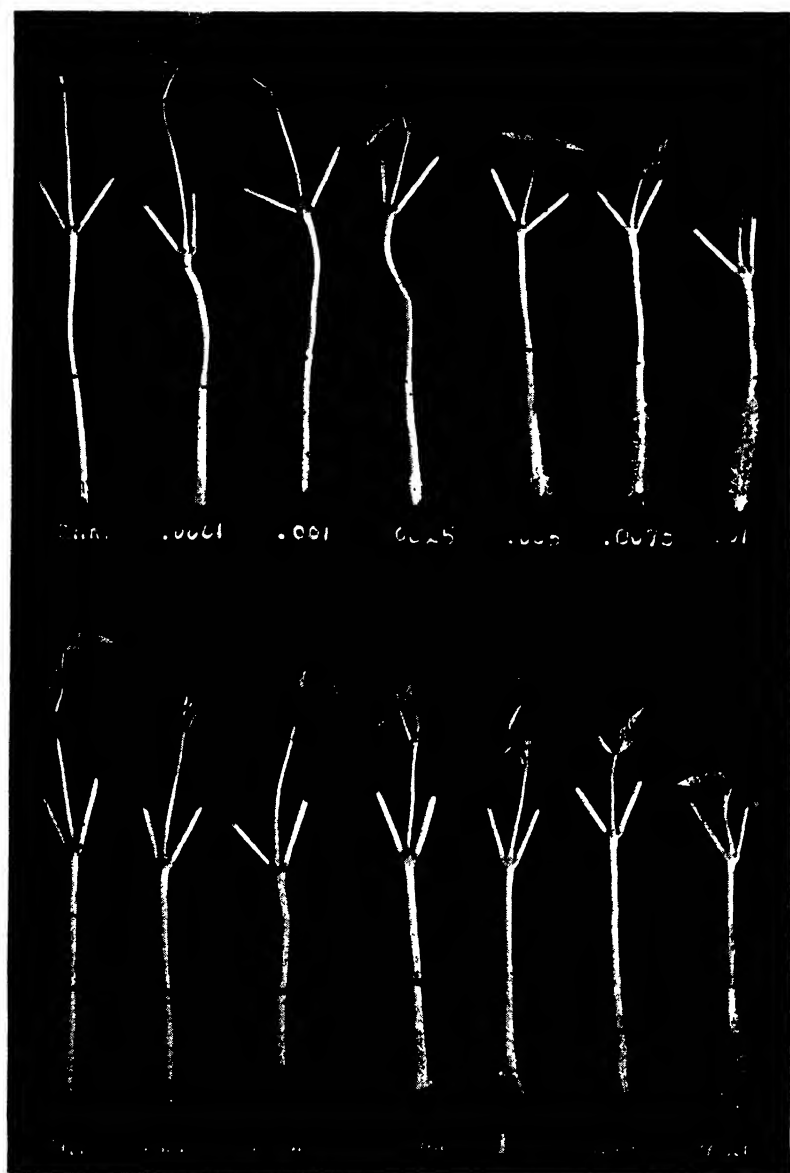


FIG. 4.—Upper: response of cuttings treated 4 hours with increasing concentrations of indoleacetic acid, 116 hours after treatment. Note progressive suppression of top growth. Lower: response when treated with 0.01 per cent indoleacetic acid for increasing lengths of time, 116 hours after treatment.

node and petioles from the control cuttings grew much more than those of the treated ones. In both treated and untreated cuttings the dry weight of the leaf blades decreased but the decrease was greater in the leaves from the treated cuttings. The results reported in table 1 are from cuttings in which the leaf area was restricted to the primary leaves and the cotyledons removed when the cuttings

TABLE 1

KIDNEY BEAN CUTTINGS TREATED JUNE 27 WITH 0.01 PER CENT INDOLE-ACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER

CUTTINGS SET IN SAND AFTER TREATMENT

HOURS AFTER TREAT- MENT	HYPOCOTYLS						FIRST INTERNODE		PETIOLES		BLADES		TOTAL	
	LOWER		UPPER		TOTAL									
	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.
DRY WEIGHT (GM.) OF 100 CUTTINGS														
0.....	3 15	2 31	5 46	..	2 72	..	2 07	..	16 4	..	26 7	..
24.....	3 20	3 35	2 47	2 50	5 76	5 85	2 96	2 75	2 17	2 08	15 4	14 9	26 3	25 6
48.....	3 32	3 74	2 40	3 03	5 72	6.77	3 03	2 72	2.23	2 08	14 8	14 3	25.8	25 9
72.....	3 21	4 35	2 75	3 16	5 96	7 51	3 20	2 87	2 57	2 08	14 7	13 8	26 5	26 3
96.....	3 61	5 53	2 87	3 25	6 48	8 78	3 70	2 95	2 76	2.07	14.6	13 1	27.5	26 9
120.....	3 66	6 30	2 04	3 17	6 60	9.47	3 83	2 84	2 82	2.01	14 2	12 5	27 5	26 8
STARCH (MG.) IN 100 CUTTINGS														
0.....	37 8	24 9	62.7	None	Trace	None	62 7
24.....	28.6	<10	<15	Trace	28.6	Trace	None	Trace	None	Trace	None	None	28 6	Trace
48.....	34 5	21 3	25 2	<10	59 7	21 3	56 7	None	43 3	None	"	"	150.7	21 3
72.....	66 8	36 1	72 9	32.2	139 7	68.3	90 1	Trace	52 0	Trace	"	"	282.7	68.3
96.....	48 7	38.2	91 3	22 1	140 0	60 3	137.3	None	62.1	Trace	"	"	330.4	60.3
120.....	50 9	30.9	85.8	<10	136 7	30 9	121 8	None	71 9	None	"	"	330.4	30.9

were made. Substantially the same results were obtained when the cotyledons were not removed and the second internode allowed to grow (table 2). It is apparent from these tables that the treatment exerted a powerful force in altering the growth response and distribution of total solids within the cuttings.

INFLUENCE OF INDOLEACETIC ACID ON THE ABSORPTION OF WATER

The great increase in dry weight of treated over untreated hypocotyls shown in table 2 was accompanied, as might be expected, by a very great increase in the amount of water. At the time of making

TABLE 2

KIDNEY BEAN CUTTINGS TREATED MAY 23 WITH 0.01 PER CENT INDOLEACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER. CUTTINGS SET IN SAND AFTER TREATMENT. COTYLEDONS AND SECOND INTERNODES NOT REMOVED

HOURS AFTER TREATMENT	HYPOCOTYLS						FIRST INTERNODE		PETIOLES		BLADES		COTYLEDONS		TOTAL	
	LOWER		UPPER		TOTAL		CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.
	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.										
DRY WEIGHT (GM.) PER 100 CUTTINGS																
0.....	2.52	2.12	4.64	1.40	1.83	16.8	6.23	30.9
12.....	2.62	2.82	2.17	2.45	4.79	5.27	1.87	1.95	1.83	2.13	16.4	17.4	5.30	5.17	30.2	31.9
24.....	2.87	3.37	2.20	2.72	5.07	6.09	1.83	2.32	1.90	2.13	15.3	15.9	5.03	4.22	29.1	30.7
48.....	2.98	3.73	3.08	3.48	6.06	7.21	2.57	2.35	2.77	2.08	16.4	14.8	3.93	3.30	31.7	29.7
96.....	3.82	5.53	3.52	4.40	7.34	9.93	3.28	2.73	2.67	2.07	15.4	14.3	3.20	2.88	31.9	31.9
144.....	4.43	8.03	3.63	4.63	8.06	12.66	4.05	2.77	3.15	2.10	16.0	14.6	2.42	0.95	33.7	33.1
TOTAL NITROGEN (MG. IN 100 CUTTINGS)																
0.....	155	125	280	65	92	1067	205	1709
12.....	150	161	122	135	272	296	81	85	84	89	1018	1032	147	144	1600	1646
24.....	156	198	123	157	279	355	84	101	86	94	971	1013	146	104	1566	1667
48.....	147	222	150	213	297	435	102	119	113	88	956	886	98	66	1566	1593
96.....	172	330	161	288	333	618	137	165	98	83	873	752	55	42	1496	1660
144.....	174	448	149	306	323	754	161	178	121	85	861	629	50	12	1516*	1658

* Check cuttings slightly lower, owing to greater growth of second internode (discarded).

the cuttings the fresh weight of 100 hypocotyls was 62.9 gm., representing 58.2 gm. of water (92.5 per cent). Six days later the control hypocotyls weighed 72.7 gm., whereas the treated ones had increased to 155.0 gm. On a percentage basis, however, the difference in moisture content was not great, the controls having 88.8 per cent and the treated ones 91.8 per cent. In the other portions of the cuttings the percentage of moisture was always lower in the controls. However, owing to the slightly greater growth of the portions other than the hypocotyls in the controls, their total content of water was slightly greater than in the corresponding portions of the treated cuttings. Considering the whole cutting, treatment with indoleacetic acid caused an increased absorption of water over that taken up by the controls.

The total pectin content of the hypocotyls was determined in an effort to explain the greater water holding capacity due to treatment with the acid. These data show that the initial control, final control, and treated hypocotyls contained 136, 235, and 495 mg. respectively of unpurified calcium pectate.

INFLUENCE OF INDOLEACETIC ACID ON DISTRIBUTION OF CARBOHYDRATES

The first effect of treatment was to cause a rapid mobilization of sugars in the hypocotyl (table 3). At the end of 48 hours the treated cuttings contained 6.6 mg. of sugar per hypocotyl while the controls had but 3.8 mg. More than half of this increase was due to sucrose. From then until the roots had emerged, at the end of 120 hours, the treated hypocotyls steadily decreased in sugars until they contained less than the initial controls. Meanwhile the control hypocotyls slowly increased in sugars.

Sugars also steadily accumulated in the first internode of the control cuttings. Exactly the reverse response was noted in the first internode of the treated cuttings, where at the end of 120 hours not a trace of sugar remained. To a slightly less extent the same response was noted in the petioles. In the leaf blades, too, the content of sugars was always less in those from the treated cuttings. This lower sugar content may have been due partly to some depressing action of indoleacetic acid on photosynthesis. However, the loss in

dry weight of the blades, coupled with the increase in weight of the hypocotyls, makes it appear certain that sugars as well as other materials were rapidly transported out of the leaf blades. Whether this is due directly to the stimulus of the acid or is caused by the

TABLE 3

KIDNEY BEAN CUTTINGS TREATED JUNE 27 WITH 0.01 PER CENT INDOLE-ACETIC ACID FOR 4 HOURS IN COMPARISON WITH WATER
CUTTINGS SET IN SAND AFTER TREATMENT

HOURS AFTER TREATMENT	HYPOCOTYLS						FIRST INTERNODE		PETIOLES		BLADES		TOTAL	
	LOWER		UPPER		TOTAL									
	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.	CHECK	IAC.
DIRECT REDUCING SUGARS (MG.) IN 100 CUTTINGS														
0	151	...	119	270	...	186	...	128	...	466	...	1050	...
24	117	194	111	150	228	353	208	187	136	117	178	166	750	823
48	140	209	126	187	275	306	258	162	183	121	271	180	987	850
72	141	179	147	171	288	353	245	132	209	100	185	198	927	702
96	161	122	131	168	292	230	231	59	200	68	227	125	959	482
120	147	120	148	68	295	188	241	None	123	29	169	37	828	254
SUCROSE (MG.) IN 100 CUTTINGS														
0	22.4	...	20.3	...	42.7	...	54.4	...	20.1	...	141	...	258	...
24	13.8	27.5	11.0	27.0	25.7	54.5	38.2	22.3	14.3	8.3	121	121	190	206
48	40.2	138.8	47.8	96.7	88.0	235.5	72.4	58.2	36.8	20.4	127	156	324	470
72	55.9	111.4	42.4	76.2	98.3	187.6	65.8	65.4	33.4	16.8	111	46	309	316
96	54.2	84.0	51.7	39.0	105.9	123.6	85.8	26.0	35.1	15.3	114	99	341	264
120	58.6	53.6	48.2	15.2	106.8	68.8	63.2	None	42.0	13.7	102	96	315	179
TOTAL SUGARS (MG.) IN 100 CUTTINGS														
0	181	...	147	...	328	...	244	...	154	...	638	...	1364	...
24	137	231	129	195	266	426	248	218	158	131	319	306	991	1081
48	197	364	182	296	379	660	344	230	230	148	420	360	1373	1398
72	206	301	198	261	404	562	324	207	253	132	278	260	1259	1161
96	225	218	192	149	417	367	331	90	255	88	362	239	1365	784
120	216	182	199	84	415	266	317	None	174	47	292	139	1198	452

greatly increased meristematic activity in the hypocotyl is not known.

It should be noted that there was a net loss of more than two-thirds of the sugar originally present and synthesized by the treated cuttings during the 120 hours in the propagating frame. The controls showed a net loss of only 12 per cent. The initial decrease in carbohydrates from both treated and control cuttings during the first 24 hours after treatment is probably due to interference with

photosynthesis while the cuttings were being made, treated, and set in the propagating frame. Respiration losses were probably greater, too, owing to wound stimulus caused by making the cutting.

Under the conditions of this study the carbohydrate metabolism was concerned principally with the sugars. Starch tests were made on all samples, and quantitative determinations made whenever a sufficient amount was present to enable estimation by the method used (table 1). The initial cuttings contained starch only in the hypocotyls. During the period in the propagating frame, additional starch was deposited in the control hypocotyls and still greater amounts laid down in the first internode and petioles. The treated cuttings never stored starch above the hypocotyl, however, and the original amount there decreased somewhat. The carbohydrate analyses demonstrate the remarkable power of the indoleacetic acid to direct the distribution and metabolism of carbohydrates.

INFLUENCE OF INDOLEACETIC ACID ON DISTRIBUTION OF TOTAL NITROGEN

The bean cuttings used in this study contained sufficient nitrogenous reserves, exclusive of the cotyledons, to account (if calculated as protein) for more than one-third of all their dry weight. The effect of treatment was to cause a prompt mobilization of nitrogen from the leaf blades to the hypocotyl (table 2). Within 12 hours after treating, an appreciable amount of nitrogen had been translocated to the hypocotyls. After 24 hours the nitrogen content of the treated hypocotyls had increased nearly 27 per cent, while the control hypocotyls showed no change in nitrogen content. At the end of 144 hours more than ten times as much nitrogen had moved into the treated hypocotyls as into those of the controls.

In the control cuttings the same directional shift of nitrogen from the leaves and cotyledons and accumulation in other parts is noted, but without the influence of the indoleacetic acid treatment this movement was comparatively sluggish. In the treated cuttings it is apparent that, even at the end of 144 hours, rapid withdrawal of nitrogen from the leaf blades and cotyledons and its accumulation in the hypocotyls was still taking place, despite the considerable movement already accomplished.

Discussion

The prompt movement of large amounts of nitrogen and carbohydrates to the treated hypocotyls was correlated with the emergence of many more roots than appeared on the control hypocotyls. The accumulation of these food materials doubtless favored root development and meristematic activity. However, other substances as well as sugars and nitrogen may have been translocated to the hypocotyls under the influence of the treatment. In this connection, COOPER (2) has presented convincing evidence that cutting off the bases of leafless woody cuttings a short time after treating them effectually removes something which is associated with root formation. COOPER refers to this substance as rhizocaline. It would be of interest to know whether appreciable amounts of the common food materials were mobilized in his cuttings and hence lost when their bases were removed.

The temporary suppression in growth of the second internode of bean cuttings was probably caused in part by the movement of carbohydrates and nitrogen away from the apical regions. The comparative subsequent growth of treated and control cuttings after rooting is now under investigation.

Various other plant responses to the application of indoleacetic acid, including such phenomena as bending, gall formation, and even induced parthenocarpy, can possibly be explained through the action of the acid in mobilizing various materials to the region of application. The results obtained in this study help to explain also the striking morphological and histological changes in bean plants treated with indoleacetic acid as reported by KRAUS and co-workers (3).

The mechanism by which the indoleacetic acid so profoundly affects the movement of food materials is unknown. THIMANN and SWEENEY (6) reported that low concentrations of indoleacetic acid accelerated the rate of protoplasmic streaming. It is not known to what extent the treatment used in the present study may have affected the transport of food materials by streaming. Further investigation is necessary before it is known whether the mobilizing action of the growth substances is due to the new meristems which

arise or to the direct action of the growth substances themselves, or to some other reaction not yet recognized.

Summary

1. Cuttings of kidney bean seedlings were treated by immersing their bases in 0.01 per cent indoleacetic acid for 4 hours. They were then set in sand in a propagating frame for 120 hours. During this period, in comparison with control cuttings, the treatment brought about a directional shift of large amounts of nitrogen and carbohydrates from the leaves and cotyledons to other portions of the cuttings, principally to the treated hypocotyls.

2. Accompanying this mobilization of materials, the treated cuttings responded by rapid swelling of the hypocotyls, profuse root production, and a temporary suppression of top growth. Total dry weight of the treated cuttings at the end of the rooting period was slightly less than that of the controls.

3. The responses were proportional to the length of exposure and the concentration of the acid.

4. The possible importance of indoleacetic acid as a mobilizer of food material in various other growth responses is suggested.

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THE AMERICAN SUGAR MAPLES
I. PHYLOGENETIC RELATIONSHIPS, AS DEDUCED
FROM A STUDY OF LEAF VARIATION

EDGAR ANDERSON AND LESLIE HUBRICHT

(WITH FIVE FIGURES)

Introduction

For a number of reasons it seemed probable that the sugar maples of eastern North America (*Acer saccharum* in the broad sense) would provide interesting material for a study of variation. In the first place, the relationships of the various elements within these maples are complex enough to be interesting (table 1), but simple enough to give some hope of eventual solution and exposition. In the second place, sugar maples usually grow in large, fairly conspicuous groves, and it is therefore possible to locate and study representative populations with a minimum of effort. Finally, and of particular importance, a considerable proportion of the characters used in specific delimitation is represented in the leaves, so that a study limited mainly to leaf variation might be expected to yield significant data. Most of these characters are resolvable into lengths or angles and are therefore easy to record objectively and to treat statistically.

Methods

Collections of leaves, one from each tree, were made in various large groves as opportunity offered. Since the general problem was more concerned with differences between trees than with differences on a single tree, much of this extraneous variation was removed by a careful selection of comparable material. Collections were not made until after midsummer, when leaves were fully mature. So far as possible, leaves were collected only from mature or nearly mature trees, from lower branches, never from fruiting branchlets, never from branches in deep shade, and never from terminal twigs. Most students realize the varying effects of such factors; the problem has

been given thorough statistical treatment by JENTYS-SZAFEROWA (4) in the case of *Betula* leaves.

Each collection was provided with a name, usually geographical. The leaves were pressed like ordinary herbarium specimens but were left unmounted. After they were completely dry, the name of the collection and the number of the leaf were written on the lower surface in pencil. Each leaf was then scored for the characters diagrammed in figure 1. The degree of pubescence was also scored, the following grades being recognized:

- A. Axils of primary veins on lower surface of leaf
 - o. Glabrous or essentially so
 - 1. Leaf visible through pubescence
 - 2. Leaf not visible through pubescence.
- B. General lower surface of leaf—
 - o. Glabrous or essentially so
 - 1. Covered with scattered hairs; not interlacing
 - 2. Covered with interlacing hairs.
- C. Petiole—
 - o. Glabrous or essentially so
 - 1. Pubescent only at junction with leaf
 - 2. Pubescent entire length.

Observations

As soon as a few representative populations had been studied, the complexity of the relationships of the various elements within the sugar maples became apparent. In each grove studied there was conspicuous variation from tree to tree. The collections from New England presented the simplest picture. The outlines of ten leaves chosen at random from the collection made at Petersham, Worcester County, Massachusetts, are illustrated in figure 2. Although there is considerable difference from leaf to leaf the variation is centered about a single plexus, and any one of these leaves could confidently be identified as belonging to *Acer saccharum* in the narrow sense.

The collections from southern Michigan were slightly more complex. The variation in these groves centered about two plexi, one

of which was practically identical with the *A. saccharum* of New England; the other was the element which we shall refer to *A. saccharum* var. *nigrum*. When the groves from southern Michigan were examined as wholes, it was evident that the variation between these two plexi was practically discontinuous. For example, of the forty-eight trees examined in the Nesbit grove, near Schoolcraft, Kalamazoo County, Michigan, thirty-one were definitely *A. saccharum*, fif-

TABLE 1

COMPARISON OF THREE TAXONOMIC TREATMENTS OF NORTHERN SUGAR MAPLES. HORIZONTAL DOTTED LINES SEPARATE VARIETIES; UNBROKEN LINES SEPARATE SPECIES

GRAY'S MANUAL	TRELEASE	REHDER'S MANUAL
<i>A. saccharum</i> Marsh	<i>A. saccharum</i> Marsh	<i>A. saccharum</i> Marsh
		<i>A. saccharum</i> var. <i>glaucum</i> Pax.
	<i>A. saccharum</i> var. <i>barbatum</i> Michx.	<i>A. saccharum</i> var. <i>schneckii</i> Rehd.
<i>A. saccharum</i> var. <i>nigrum</i> (Michx. f.) Britton		<i>A. saccharum</i> var. <i>rugelii</i> (Pax.) Rehd.
	<i>A. saccharum</i> var. <i>nigrum</i> (Michx. f.) Britton	<i>A. nigrum</i> Michx. f.
		<i>A. nigrum</i> var. <i>palmerii</i> Sarg.

teen belonged to *A. saccharum* var. *nigrum*, and two were apparently intermediate.

The problem was made more complex by the inclusion of collections from Missouri and southern Illinois. The results of these collections were not only difficult to harmonize with those from the north; they did not even agree with each other. At some localities it was possible to divide the collections into two plexi, one of which was apparently the same as *A. saccharum* of Michigan and Massachusetts, although it differed more from them than they did from each other. The second was apparently *A. saccharum* var. *nigrum*, although it presented certain anomalies. In other groves no such discontinuity was perceptible. At such localities there were some

trees very similar to those of *A. saccharum* in Michigan and New England; there were other trees which on the basis of a single specimen might have been identified with *A. saccharum* var. *nigrum*; but there were other types not seen in the north, and the whole collection

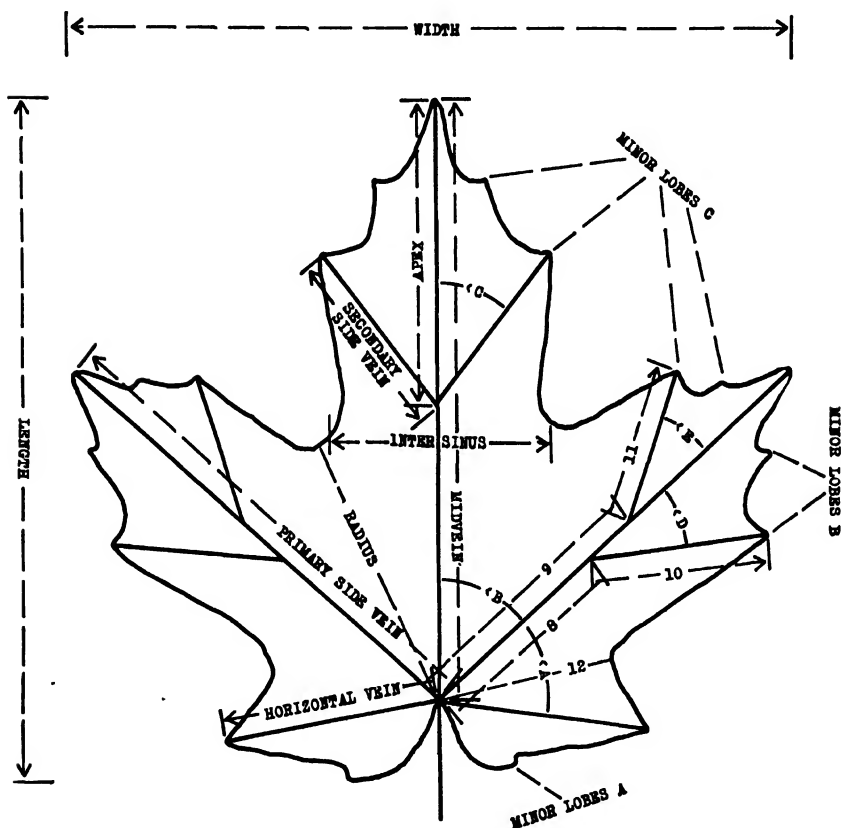
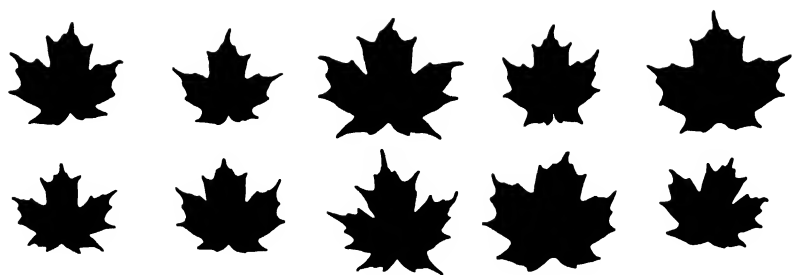
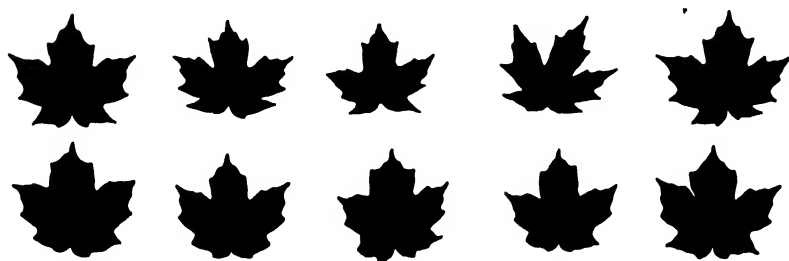


FIG. 1.—Maple leaf showing measurements and enumerations used

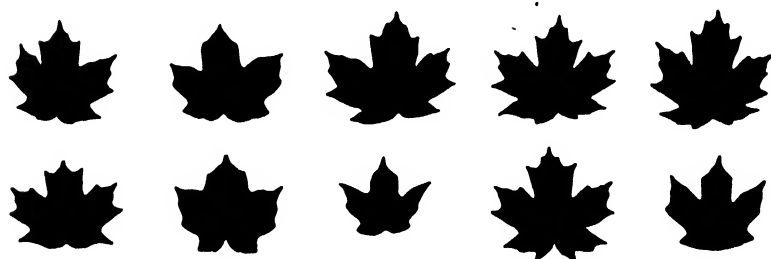
was so intricately related morphologically that no separate or even partially separate elements could be distinguished. The grove could be considered as a whole or as individual trees, but no other natural groupings of the material could be made. The outlines of ten leaves selected at random from the collection made at Fountain Gap, Monroe County, Illinois, will show something of the great variation encountered at such localities (fig. 2).



PETERSHAM, MASS.



SCHOOLCRAFT, MICH.



FOUNTAIN GAP, ILL.

FIG. 2.—Variation between individuals. Outlines of representative leaves from ten trees at Petersham, Massachusetts (all *A. saccharum*), ten at the Nesbit grove near Schoolcraft, Michigan (five *A. saccharum* and five *A. saccharum* var. *nigrum*), and ten from Fountain Gap, Illinois (various species).

It is not the purpose of this paper to discuss these complexities. For the most part they have been adequately treated in such papers as that by TRELEASE (6). It is rather our purpose to show how a combined quantitative and qualitative study of the variation in these maples may lead to working hypotheses as to their relationships, and suggest new lines of attack.

There are two kinds of evidence in such problems, which are gleaned more reliably from the study of entire populations than from the study of single individuals. The first of these is an estimate of the discontinuity of variation. In studying sugar maples, for instance, a much more accurate reflection of the amount of intercrossing between *A. saccharum* and *A. saccharum* var. *nigrum* can be gained from a study of a population in which they both occur than from the usual herbarium material.

The second sort of data more reliably gained from whole populations is averages and their comparison. One of the chief advantages of quantitative characters is that they can be averaged and compared. On the other hand, averages of isolated characters are not particularly useful in discriminating between species, varieties, or races (1). Much more significant is the average of each character in relation to the average of all the other characters. An attempt was therefore made to turn the averages for each grove into a picture of what might be called the average leaf of that locality. The averages of the twenty-two measurements illustrated in figure 1 provided, when combined, the skeleton of such a leaf, with the main veins, sinuses, and apices determined, but without a margin. To complete the average leaf it was necessary to provide some kind of average margin. A working approximation was obtained in the following manner. Margins of sugar maple leaves differ in the number of serrations between apices, and in the curves of the actual margin. The margin was divided into six arcs on each side, marked by the apices and sinuses (fig. 1). For each of these arcs the average number of major serrations was calculated. To obtain an average value for the undulations of the margin within each arc, a tracing was made of that arc for each leaf. These tracings were then superimposed on the same base line and with the same point of origin (fig. 3). Knowing the average length and the average number of serrations, it was

comparatively easy from this composite tracing to draw, by inspection, a modal margin for each arc. These were then transferred to the average skeleton.

Average leaves prepared in this manner are shown in figure 4. Of particular significance are the averages for *A. saccharum* from Peter-

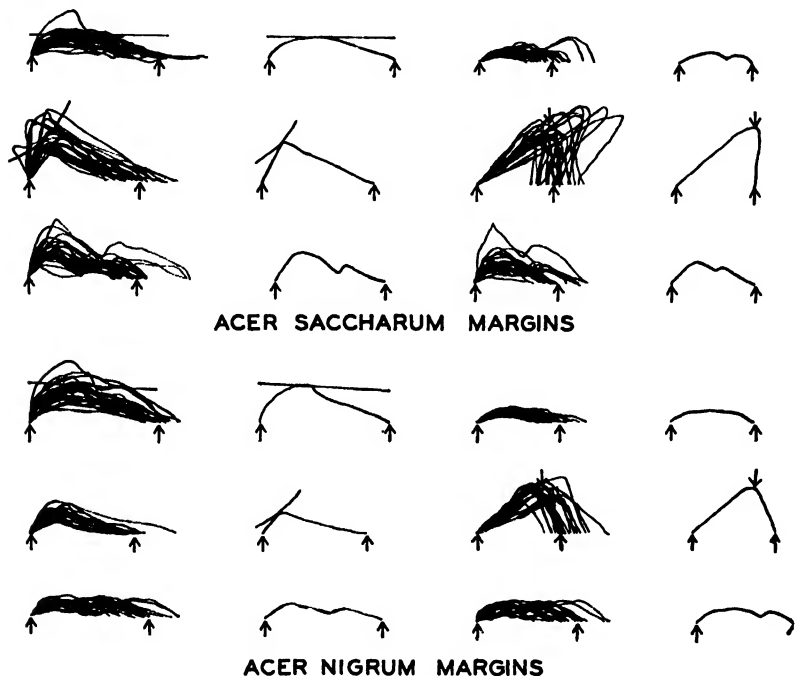


FIG. 3.—Graphical method for obtaining average of leaf margin; further explanation in text.

sham and from the Nesbit grove. Whatever their relationship nomenclatorially or phylogenetically, *A. saccharum* and *A. saccharum* var. *nigrum* are very similar. Although they are often found growing intermingled, flowering and fruiting at the same times (in the north at least), they remain distinct in spite of their obviously close relationship. Morphologically the total hiatus between them, in proportion to the variation within either element, is only a fraction of that usually found between closely related species. Unless the internal barrier is very powerful indeed, we should expect to find that *A. sac-*

charum growing in the same groves with *A. saccharum* var. *nigrum* would on the average be more *nigrum*-like than when it grows alone. The Petersham and Nesbit averages show no evidence of such an effect. They support the opinion of such students as L. H. BAILEY (2), who found that, in spite of their remarkable similarity and frequent juxtaposition, these two maples remain entirely distinct throughout much of their range.

To a cytogeneticist, these facts suggest a simple working hypothesis as to phylogenetic relationships. If the germplasm of *A. saccharum* var. *nigrum* were practically that of *A. saccharum* plus something else, and if this something else were added in such a way as to create an effective barrier to intercrossing (inversions, polyploidy, etc.), then we should expect just such a relationship. The something else would at the same time create a barrier to intercrossing and produce a slight but definite morphological hiatus between the original stock and its derivative variety. The preceding averages may be used to show what a maple would look like which could cause such an interaction with *A. saccharum*. On our hypothesis: *A. saccharum* var. *nigrum* = *A. saccharum* + x . The morphological relationships between these three elements should be as follows:¹

$$\frac{A. saccharum}{A. saccharum \text{ var. } nigrum} = \frac{A. saccharum \text{ var. } nigrum}{x}$$

Since only one of these terms is unknown, it is a simple matter to solve the equation. If we determine statistically the appropriate lengths and angles for x and assemble them into a composite, we have the maple shown in figure 4.

We have thus obtained by statistical prediction the kind of a maple which by interaction with *A. saccharum* might produce such a maple as *A. saccharum* var. *nigrum*. The prediction is interesting since such a maple does exist and in eastern North America. It is in fact the *Acer rugelii* or *A. saccharum* var. *barbatum* of table 1. We have ourselves collected it frequently in the edge of the Ozarks; it is

¹ The actual value to be assigned to x would depend upon the relative proportions of x and *A. saccharum* in the germplasm of *A. saccharum* var. *nigrum*. Since as yet we have no information on that point, the calculations have been made on the simplest possible assumption: equal amounts of each. This would of course be the expected proportions for an amphidiploid relationship.

actually one of the complexities met with in such groves as that at Fountain Gap. Figure 5 shows how our predicted leaf is almost identical with one of the illustrations of *A. saccharum* var. *barbatum* published by TRELEASE in 1894.

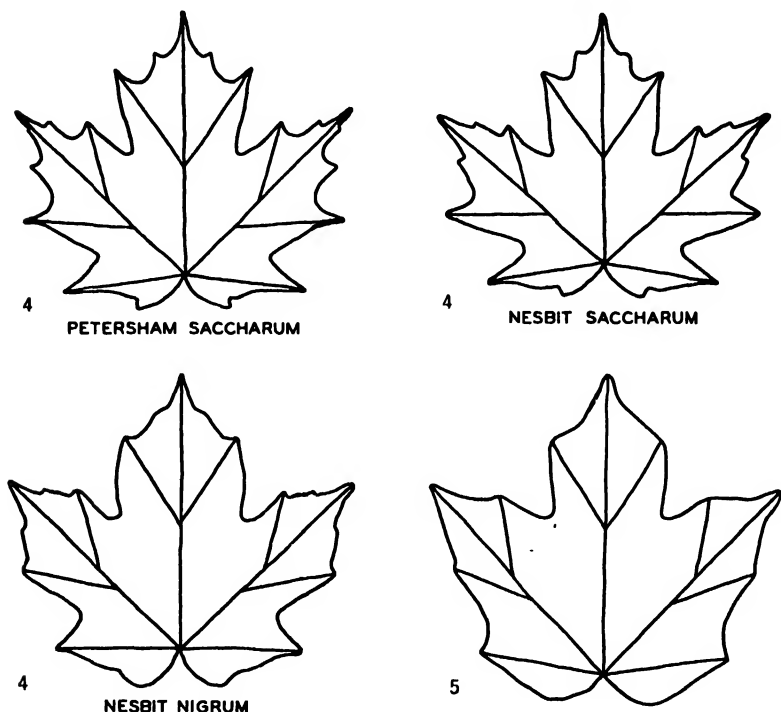


FIG. 4. -Average leaves for Petersham grove and for *A. saccharum* and *A. saccharum* var. *nigrum* in Nesbit grove. Lower righthand leaf (5) is a hypothetical one, determined by statistical prediction, for comparison with fig. 5, opposite page.

As a basis for further experiment, we therefore advance the hypothesis that *A. saccharum* and *A. saccharum* var. *rugelii* are the older elements in the North American sugar maples, and that *A. saccharum* var. *nigrum* has been derived from them, in some way as yet undetermined.

Summary

1. *Acer saccharum* (in the broad sense) is promising material for the study of variation, since: it presents an interesting but compara-



FIG. 5.—Reproduction of TRELEASE's original drawing of *A. saccharum* var. *barbatum* (used by permission), for comparison with hypothetical leaf (fig. 5 on opposite page) determined by statistical prediction.

tively simple phylogenetic problem; it grows in large, easily located groves; most of the specific differences are confined to the leaves; many of these differences can be recorded as lines and angles and are then readily treated statistically.

2. Methods of collecting and measuring leaves are described.

3. Study of such collections shows: (a) The New England groves, while variable, centered around a single plexus,² *A. saccharum* (in the narrow sense). (b) The groves from southwestern Michigan centered around two almost discontinuous plexi, *A. saccharum* and *A. saccharum* var. *nigrum*. (c) The groves from southern Missouri and southern Illinois were sometimes like those from Michigan. Other groves from this region showed great variability but no distinguishable variation centers.

4. A method for reconstructing an average leaf from each population sample is described.

5. A study of such average leaves shows that *A. saccharum* var. *nigrum* had no effect on *A. saccharum*, even when they grew intermingled and flowered at the same time.

6. Possible phylogenetic relationships of *A. saccharum* and *A. saccharum* var. *nigrum* are discussed. On the hypothesis that *A. saccharum* var. *nigrum* represents *A. saccharum* modified by an unknown *Acer*, the characters of this unknown are determined by statistical prediction, and prove to be practically identical with *A. saccharum* var. *rugelii*.

7. It is therefore suggested, as a working hypothesis, that the older elements in the northeastern sugar maples are *A. saccharum* (in the narrow sense) and *A. saccharum* var. *rugelii*; and that *A. saccharum* var. *nigrum* represents some sort of interaction product between these two.

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² While *A. saccharum* var. *nigrum* does occur in certain parts of New England, it was not present in any of the groves studied.

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NOTES ON SOME PLANT REMAINS FROM THE CARBONIFEROUS OF ILLINOIS

FREDDA D. REED

(WITH NINETEEN FIGURES)

Introduction

The plant fragments here described were found in coal ball 236 of the Harrisburg collection, made at Harrisburg, Illinois by Dr. A. C. No , under the auspices of the Illinois State Geological Survey. The coal balls were found in coal seam no. 5, which is in the Alleghany group of the Upper Pennsylvanian. Although the mines have now been closed, thereby making it impossible to do further collecting in the locality, except from the mine dumps, there are still at hand a number of concretions awaiting examination.

The fragments consist of a stem tip surrounded by imbricated sheaths of leaf bases, of scattered leaves, and of some reproductive structures (sporangiophores with attached sporangia containing spores), none of which, in so far as I have been able to determine, are referable to any previously described forms from the Carboniferous of America. Scattered through the same coal ball were many other plant fragments, portions of leaves, petioles, stems, roots, sporangia, and synangia, all of them recognizable as belonging to well established Paleozoic forms. Since there are so many different genera, and since there is such diversity of both vegetative and reproductive structures so heterogeneously assembled and closely associated in the concretion, the mere fact of the presence of "unknown fragments" in the coal ball would be no basis for assuming relationship among them. Moreover, in the absence of any organic connection of the fragments under consideration, it is impossible to relate them definitely. Yet an examination of their structure reveals in each case certain features that indicate a Calamitean affinity for all of them. Therefore the stem, leaves, and sporangia are described separately and in order; not with any assurance that they belong to the same plant, but with the possibility that they may have been so associated

and with the hope that additional material and subsequent investigation will determine their relationship.

Observations

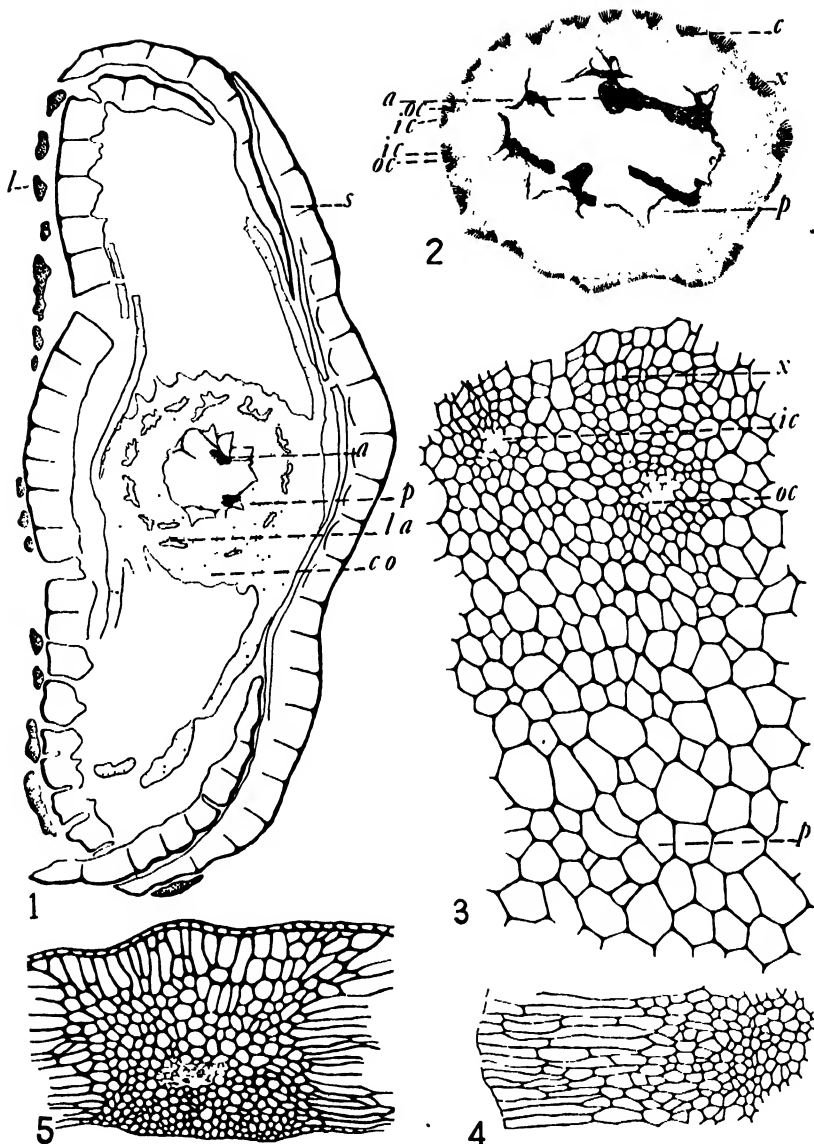
STEM

The stem fragment consists of a stem tip 3-4 cm. in length. In transverse section the pattern made by the stem and surrounding verticels of leaf bases is oval (fig. 1), but this shape may be the result of some external pressure before petrification occurred and it would otherwise be circular. The long diameter of the fragment, including the leaf bases as shown in figure 1, is 2.3 cm., and the short diameter is 1.5 cm. The diameter of the stem at the level of figure 1, which was taken near the base, is 7 mm., while toward the tip it narrows to a diameter of 3.5 mm.

The stem is hollow, with remnants of disintegrating tissue (figs. 1, 2a) extending into the fistular cavity just as sometimes may be found in modern *Equisetum* stems. The cavity is surrounded by a cylindrical mass of parenchymatous pith, which centripetally appears to be definitely organized into eleven wedge-shaped groups at the level of figure 2. The cells of the pith are relatively thick walled and vertically elongated (figs. 3, 6, 7); those toward the center are larger in transverse diameter, measuring about 70 μ . From the center outward they become increasingly smaller, until in the peripheral region they average about 20 μ (fig. 3).

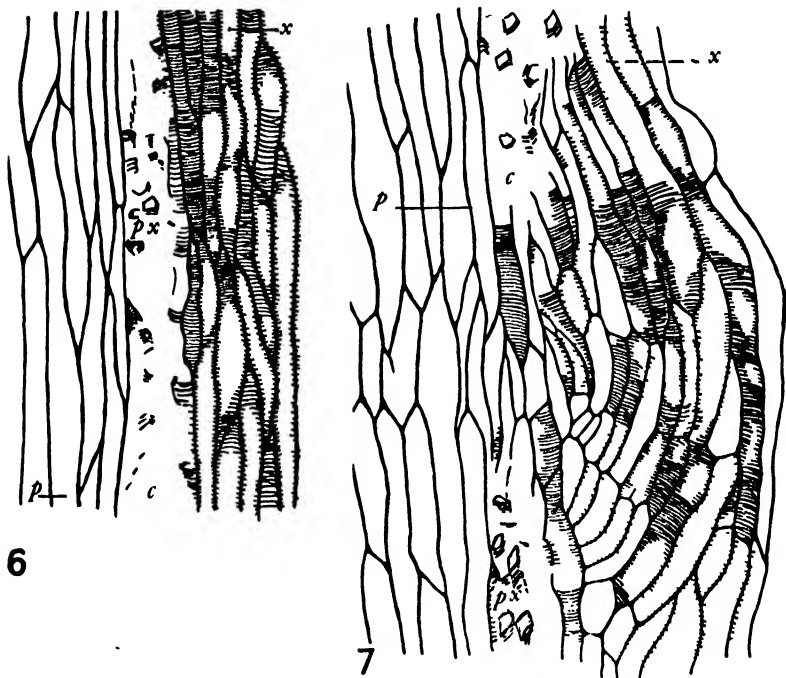
At the periphery of the pith zone are some canals from which the cells radiate centrifugally. The canals are possibly homologous with the carinal canals of *Calamites* and *Equisetum*. The cells radiating from them are interpreted as secondary wood. The canals are regularly disposed, four to each of the wedge-shaped groups; and of the four the two lateral (figs. 2, 3oc) are nearer the center than the two inner ones (figs. 2, 3ic).

In transverse section the peripheral cells, because of their size and the thickness of their walls, are indistinguishable from those of the adjacent xylem elements; limitation of the vascular strand is consequently obscure. A radial longitudinal section through one of the canals reveals the inner limit of the xylem, and indicates the method



FIGS. 1-5.—*Calamites multifolia*. Fig. 1, diagram of transverse section of stem and verticils of leaf sheaths: *l*, leaf; *s*, leaf sheath; *la*, lacunar area (probably region of phloem); *co*, cortex; *p*, pith; *a*, remnants of disintegrating pith tissue. \times about 5. Fig. 2, transverse section of stele: *x*, secondary wood; *cc*, carinal canal; *ic*, *oc*, inner and outer canals of group of four; other lettering as in fig. 1. $\times 12$. Fig. 3, detail of pith and wood of fig. 2. $\times 80$. Fig. 4, detail of portion of cortex; inner cortex to right. $\times 35$. Fig. 5, detail of segment of leaf sheath; vascular strand imperfectly preserved. $\times 35$.

of formation of the canal as well. As shown in both figures 6 and 7, the canal is bordered on the inner side by pith and on the outer side by xylem; within are traces of disorganized xylem, the most complete disorganization being found on the side next to the pith. It is evident, therefore, that the canal is the result of disintegration of



FIGS. 6, 7. *Calamites multifolia*. Fig. 6, radial longitudinal section through carinal canal; to the left is pith (*p*); in the canal are remnants of disorganizing protoxylem (*px*); to the right are scalariform tracheids of metaxylem and secondary xylem. $\times 107$. Fig. 7, longitudinal section through nodal xylem, also showing disorganizing protoxylem. $\times 107$.

xylem elements; also that the first elements, protoxylem, to break down were centripetal and that the bundle is endarch. The markings on the walls of the metaxylem and secondary xylem are scalariform to reticulate.

At the basal end of the fragment there is no structure preserved intact beyond the secondary wood (fig. 2). At a slightly higher level

the cortex is preserved yet partially separated from the woody zone by lacunar areas (fig. 11a). These lacunar areas provide the only clue to the former existence and position of the phloem, which was not preserved probably because of the soft texture of the cell walls.

The cells of the cortex show some variation; those of the inner region resemble the pith in size and structure, while those toward the exterior are radially elongated and radially arranged. The epidermis was not observed.

Toward the apical end of the fragment the tissues are progressively less mature, until at the apex there is no distinguishable organization into vascular bundles; instead the cells all appear to be parenchymatous and fairly uniform in size, with the cell walls appreciably thinner.

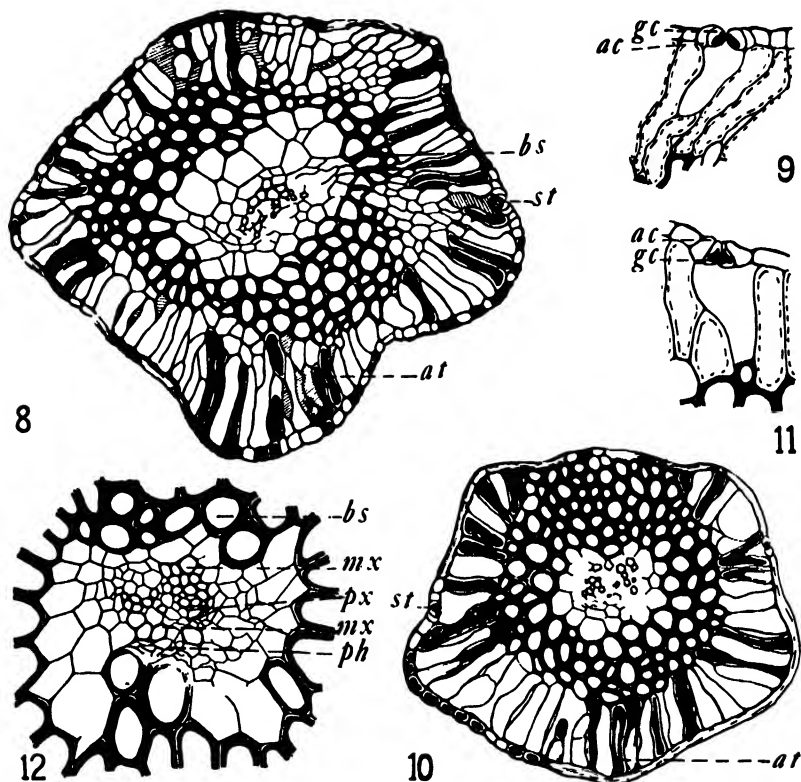
Beyond the cortex are the sheaths of leaf bases. These are somewhat crushed, are more or less completely preserved, and are partly separated by intruded fragments of other plants. Because of the incomplete preservation and fragmentation of the verticels of leaf sheaths, a detailed account of their number and structure is impossible. At the level of figure 2 there appear to be portions of two whorls, whereas at the tip there are four or five. When the sheath diverges from the stem it is composed of some forty coherent segments which at a higher level separate into as many discrete units, the leaves (fig. 11). Each segment of the sheath is provided with a vascular strand.

LEAF

In almost every section of coal ball 236 there are portions of leaves variously cut which in their general features resemble those of *Calamites* (3). Some of the transverse sections are almost completely preserved, making it possible to work out the anatomical detail from that aspect. Unfortunately the longitudinal sections are not so satisfactory.

There is considerable range in the variability of the shape of the transverse sections of the leaves: most of them are roughly rectangular with a slight median ridge on the adaxial side and a convex abaxial surface (fig. 8); some of the leaves are more regularly rectangular than the one illustrated in figure 8; and an occasional one

may be found of the proportions shown in figure 10. The variation is in the degree of development of the several tissues rather than in any specific difference in organization, and the range of variability lies well within the limit found in different leaves of the same plant,



FIGS. 8-12.—*Asterophyllites (Calamocladus) multifolia*. Fig. 8, transverse section of leaf: *st*, stoma; *at*, assimilating tissue; *bs*, bundle sheath. $\times 80$. Fig. 9, stoma from fig. 8: *gc*, guard cell; *ac*, accessory cell. $\times 160$. Fig. 10, transverse section of leaf in which bundle sheath extends to epidermis on adaxial side. $\times 80$. Fig. 11, detail of stoma of fig. 10. $\times 160$. Fig. 12, vascular bundle of leaf: *px*, protoxylem; *mx*, metaxylem; *ph*, phloem. $\times 155$.

or even within the limit that might be found in one leaf if it were possible to secure all the transverse sections from the base to the apex.

The leaves average about 0.8 mm. wide by 0.45 mm. thick. The

length was not determined, but one leaf followed through a distance of 5-6 mm. showed no diminution in width nor gave any indication of its extremities.

The epidermis is not irregularly cutinized, as has been found for some of the Calamitean leaves (3). In transverse section the epidermal cells are relatively small and are rectangular to square, many of them filled with dark brown contents. Only rarely are the stomata found, but their seeming sparseness can probably be attributed to the imperfection of preservation of the epidermis and to their very small size, which renders them obscure except under high magnification. Those observed were on the edges of the leaf (figs. 8, 10). The guard cells are on the same level or only slightly below the level of the other epidermal cells, are somewhat smaller than the surrounding accessory cells, and contain dark opaque material. The stomata open into the intercellular spaces of the assimilating tissue.

Under the epidermis is the assimilating tissue composed of a single layer of elongated palisade-like cells and many intercellular spaces. In some sections (fig. 8) these palisade-like cells form a continuous layer under the epidermis, but more often the layer is interrupted on the adaxial side by fibrous cells of the bundle sheath (fig. 10). Frequently the cells are filled with dark organic material. Interiorly the assimilating tissue abuts the bundle sheath.

The bundle sheath is a conspicuous feature. As compared with the entire section it is a massive tissue made up of several layers of large, thick walled cells, isodiametric in cross section and much elongated in longitudinal section. Some of the cells are filled with light or rarely dark brown material but most of them lack any contents. In some of the sections (fig. 10) the sheath is seen not only as a continuous layer about the vascular strand, but on the adaxial side it is expanded and extends to the epidermis.

The vascular bundle occupies the center of the leaf. Figure 12 represents a bundle in which the preservation is almost perfect and shows the distribution of the various elements. Toward the center are lignified cells of small caliber, the protoxylem, surrounded by metaxylem interspersed with parenchyma. By far the greater development of the metaxylem is on the adaxial side; that is, it is centripetal. Between the centrifugal metaxylem and the bundle

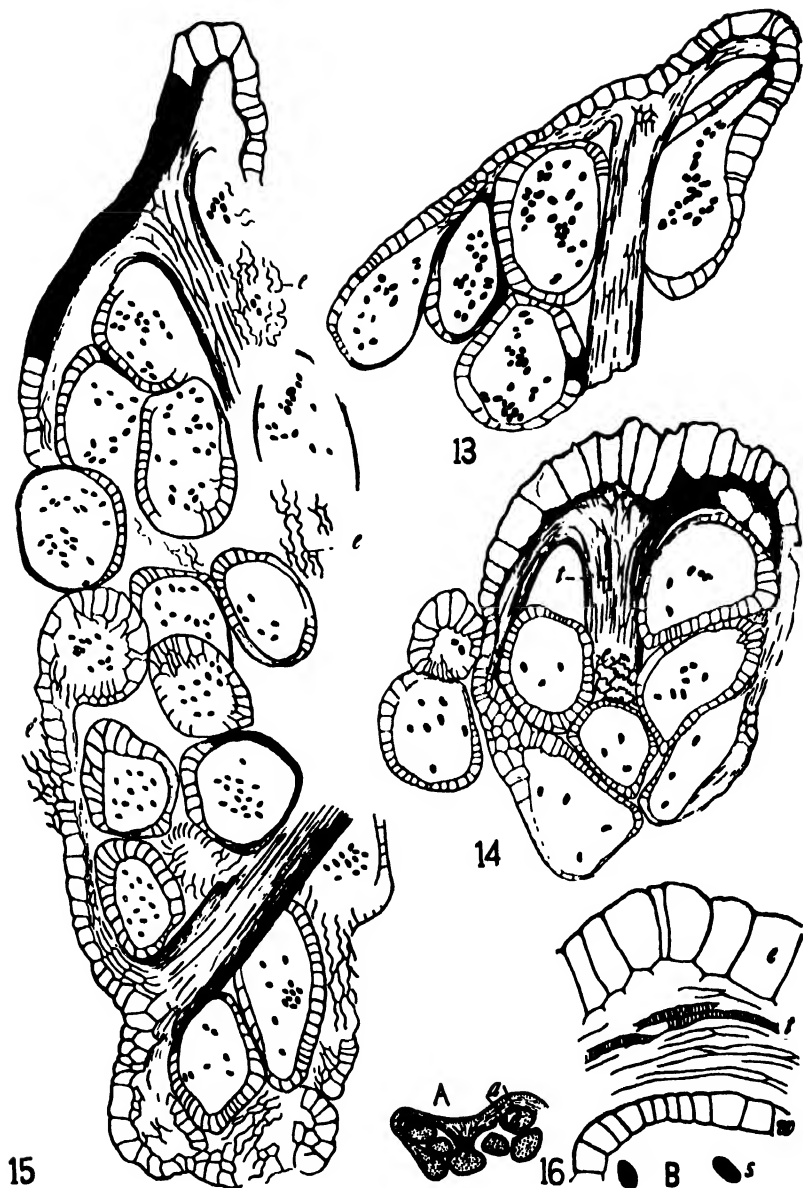
sheath are a few layers of thin walled, irregular, and somewhat crushed cells which doubtless constitute the phloem; the bundle is therefore interpreted as being collateral and not concentric, as THOMAS reported for *Calamites* leaves (3). Surrounding the conducting strand but chiefly developed laterally are thin walled parenchymatous cells.

SPORANGIA

As almost every section of the coal ball contained leaves of the type just described, so also on almost every section might be found groups of sporangia, sometimes few in a cluster and sometimes many, some apparently detached and isolated and others still in connection with the sporangiophore. The position of the sporangia with reference to the sporangiophore and the juxtaposition of two of the sporangiophores (fig. 15) strongly suggest an original aggregation of these organs into a cone. The peltate top of the sporangiophore and the mode of attachment of the sporangia are Calamitean and Equisetalean.

The sporangiophore consists of a long stalk which expands distally into a peltate top. The edge of the peltate top is curved under and divided into lobes, each lobe more or less extended and bearing on the end a single sporangium (figs. 13, 14). The number of lobes and corresponding number of sporangia attached to a single sporangiophore were not definitely determined, but following through a single cluster for little more than half its thickness eighteen sporangia were counted. Twenty-five to thirty sporangia to a sporangiophore, therefore, would be a rough but conservative estimate.

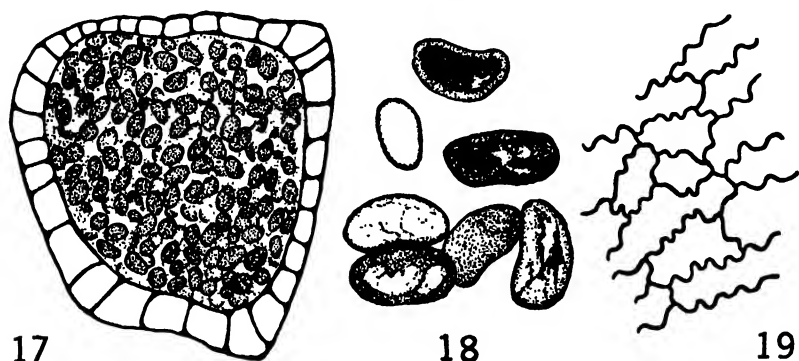
The stalk is composed of narrow, elongated, thick walled cells inclosing a central vascular strand. In section the upper epidermis of the scale presents a columnar or palisade-like layer, underneath which the cells are smaller and radially elongated; in this last tissue may be found tracheids which are directed toward the sporangia (fig. 16). The scale is thickest toward the center and gradually tapers to the edge, where it narrows to the two epidermal layers with few, perhaps not more than three or four, cells in between (fig. 14); then it is as if the two layers expand again and become the wall layers of the sporangia (figs. 13, 14, 15). Surface views of the epi-



FIGS. 13-16.—Figs. 13, 14, 15, sporangiophores and sporangia: *e'*, surface view of few of epidermal cells of peltate top of sporangiophore; *e*, surface view of epidermal or wall cells of sporangia. $\times 35$. Fig. 16, *A*: diagram of sporangiophore and sporangia. *B*: detail from *a-a* of *A* showing: *l*, vascular elements of peltate top leading to sporangium; *e*, epidermis of peltate top; *w*, wall of sporangium; *s*, spores. $\times 95$.

dermal cells are sometimes found (figs. 15, 19); except for their position it would be difficult to distinguish between the epidermal cells of the scale and a surface view of the cells composing the wall of the sporangium (fig. 15).

The sporangia are round to angular in section (figs. 13, 14, 15), the latter character obviously resulting from their large number and the compact arrangement. As preserved, the sporangial wall is but one cell thick with no trace of tapetal tissue. The spores are elongate-



FIGS. 17-19. --Fig. 17, section of sporangium with spores. $\times 94$. Fig. 18, detail of spores. $\times 366$. Fig. 19, surface view of peltate top of sporangiophore. $\times 94$.

elliptical, $50\ \mu$ long and about $25\ \mu$ wide. The spore coats have no characteristic markings. They are smooth save for a slight wrinkling, and are devoid of contents.

Discussion

In the stem fragment the carinal canal, centripetal protoxylem, and verticels of leaf sheaths make a combination of characters found among Paleozoic plants only in the Calamitaceae. The arrangement of the secondary wood and rays is conformable with that of *Calamites* (2). Grouping of carinal canals in pairs has been reported in the axis of the strobilus of *Palaeostachya vera*, a cone referred to *Calamites* (1); yet the particular type of grouping found in this specimen is a new feature. In addition, the relatively small size and inconspicuousness of the carinal canals, together with the massive pith consisting of thick walled, almost fibrous, cells, are features

specific to the specimen. These are of sufficient importance to warrant the establishment of a new species, for which the name *multifolia* is proposed.

Calamites multifolia sp. nov.

SPECIFIC DIAGNOSIS.—Stem hollow with fistular cavity surrounded by extensive pith of relatively thick walled cells which toward the center is arranged in wedge-shaped groups. Carinal canals between forty and fifty, small and inconspicuous, disposed in groups of four with the two lateral canals of each group on shorter radii than the ones in between.

In the kind and distribution of their fundamental tissues there is such close agreement in the leaves just described with those of the *Asterophyllites* (*Calamocladus*) type that there seems no doubt of their generic position. In the detail of structure, however, there are specific characters, consisting in the amount of development of the several tissues and in the arrangement of vascular elements, which serve to set these leaves apart into a separate species for which the name *multifolia* is again employed.

Asterophyllites (*Calamocladus*) *multifolia* sp. nov.

SPECIFIC DIAGNOSIS.—Leaves acicular, 0.8 mm. wide by 0.45 mm. thick, more than 6 mm. long. In transverse section roughly rectangular. Center traversed by a single collateral mesarch bundle surrounded by a bundle sheath of fibrous cells which on the adaxial side may extend to the epidermis. Assimilating tissue of elongated palisade-like cells and intercellular spaces. Stomata lateral and superficial.

The fragmented condition of the sporangiophores and the fact that there is only an indication of their aggregation into a strobilus make their assignment to any known fructification impossible. The peltate scale and the origin and attachment of the sporangia are definitely Equisetalean; but the large number and small size of the sporangia are at variance with any of the recorded genera of extinct forms, in which the usual number of sporangia attached to a sporangiophore is four, which extend from the peltate scale to the axis of the strobilus. With the information at hand, however, it does not

seem wise to establish a new genus for the reception of these clusters of sporangia; hence they are simply recorded as new fructification fragments possessing Calamitean characters.

Summary

1. Fragments of a stem, leaves, and sporangiophores bearing sporangia containing spores, all hitherto undescribed species and all possessing Calamitean characters, were found in coal ball 236 of the Harrisburg, Illinois, collection. The geological age of the material is the Alleghany group of the Upper Pennsylvanian.

2. In its anatomical structure the stem is conformable with the genus *Calamites*, but varies in detail from any of the described species, and so the specific name *multifolia* has been assigned to it.

3. Many sections of Calamitean leaves were found, some of them remarkably well preserved. In general they resemble leaves of the *Asterophyllites* type of the British Coal Measures, yet differ from them in specific details to such an extent that they too have been assigned to a new species, *multifolia*.

4. The sporangiophores are all detached from any vegetative structure and are separated from one another, except in one instance where two of them were found so related as to suggest an original aggregation into a strobilus. They are unique in the number (twenty-five to thirty) of sporangia they bear. The sporangia are round to angular in section, with a single layer of wall cells, and contain numerous spores. The spores are elliptical to elongate, $25 \times 50 \mu$, with smooth spore coats and devoid of contents.

5. It is suggested that the fragments described may possibly be vegetative and reproductive portions of the same plant.

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NITROGEN NUTRITION AND NICOTINE SYNTHESIS IN TOBACCO¹

RAY F. DAWSON

(WITH ONE FIGURE)

Introduction

The influence of nitrogen nutrition on the synthesis of nicotine constitutes an important aspect of the physiological relationships of this alkaloid in the tobacco plant. THATCHER, STREETER, and COLLISON (9), and GARNER *et al.* (7) have shown that the nicotine content of field-grown plants increases with increased application of nitrogenous fertilizers. Likewise the relation of form of nitrogen to the nicotine content and quality of tobacco leaf has been studied by BAILEY and ANDERSON (3), and GARNER *et al.* (7). BEAUMONT *et al.* (4) have pointed out the lack of uniformity in results obtained in studies of the latter type, and have presented data to show that ammonium nitrogen in water culture solutions induced a higher concentration of nicotine in the leaf tissues of Havana Seed tobacco than did nitrate nitrogen. To the extent that it seems to bear on the problem of nicotine formation in the plant, this observation invites further investigation.

In the present study, the influence of ammonium, nitrate, and urea nitrogen was determined upon the nicotine content of tobacco leaves. Most of the studies thus far reported have been conducted in the field under favorable light conditions. The present investigation was conducted under opposite conditions, in which light may have been a limiting factor through its effects upon carbohydrate synthesis; that is, the plants were grown and harvested during that portion of the year in which the sun's rays are normally reduced in intensity and altered in quality. The effects of the shortened photoperiod were avoided to some extent by the use of supplementary artificial illumination. Under such conditions, between October 7,

¹ Contribution from the Osborn Botanical Laboratory, Yale University. The writer wishes to acknowledge the helpful advice and criticism of Professor CARL G. DEUBER.

1936 and March 28, 1937, three crops of tobacco were grown in the greenhouse in sand cultures provided with continuously flowing nutrient solutions.

Investigation

METHOD OF CULTURE.—The technique of sand culture with continuously renewed nutrient solutions as developed by SHIVE and co-workers (5) for use with the tomato plant was adapted for the present study. Six to 8 weeks old seedlings of the desired variety of tobacco were transplanted to glazed stoneware crocks, each of which contained 2.2 liters of clean-washed white quartz sand of 42 per cent porosity. Each crock contained a hole in the bottom for drainage. Nutrient solution dripped from capillary tubes into small glass wells imbedded in the sand to prevent surface growth of algae. Three such capillary tubes were connected to one solution reservoir by a siphon so arranged that a constant head of water was maintained regardless of the level of the solution in the reservoir. Twice daily the drip rate was adjusted by means of screw clamps. Ordinarily the rate of flow was regulated between one and two liters per day, depending upon the size of the plants and the changes in pH of the outgoing solution.

Usually, twenty-four plants were grown under these conditions for periods ranging from 5 to 6 weeks, during which time the pH of the solutions did not vary over ± 0.2 . In all, four culture solutions were employed, three of which were identical with those employed by CLARK (5) in his study of the tomato. The fourth solution was molecularly equivalent to the nitrate solution of CLARK but contained instead organic nitrogen in the form of urea. The partial volume-molecular concentrations of the various solutions are given in table 1. Iron was supplied by scattering crystals of iron potassium tartrate upon the surface of the sand at weekly intervals. Manganese, boron, and copper were added in concentrations of two parts per million, and unless otherwise stated, each solution was adjusted to a reaction of pH 6.8 with sodium hydroxide. All solutions were made up with tap water from the New Haven water supply. Analyses furnished through the courtesy of the New Haven Water Company indicated that this water contained approximately 22 parts of calcium, 13 parts of sulphate, and 1.6 parts of nitrate per million.

The entire assembly of plants and apparatus was placed upon specially constructed wooden platforms on the north bench of the greenhouse. In order to counteract the effects of occasional bright days, which sometimes caused wilting, a white cheesecloth shade was erected under the roof of the greenhouse and allowed to remain throughout the duration of the experiments. The cloth was of a thin grade with a coarse mesh, which gave uniformly diffused illumination, while, according to readings obtained with a Weston light meter, it was effective in decreasing the light intensity by nearly 35

TABLE 1

COMPOSITION OF SOLUTIONS EMPLOYED IN SAND CULTURE EXPERIMENTS

SALT	PARTIAL VOLUME-MOLECULAR CONCENTRATION			
	NITRATE	AMMONIUM (CONCENTRATED)	AMMONIUM (DILUTE)	UREA
Calcium nitrate.....	0.0042*
Potassium monophosphate..	0.0021	0.0021	0.0021	0.0021
Magnesium sulphate.....	0.0042	0.0042	0.0042	0.0042
Ammonium sulphate.....	0.0042	0.0021
Calcium chloride.....	0.0042	0.0042	0.0042
Urea.....	0.0042

* The final partial molecular concentration of the nitrate solutions was later discovered to be less than this value, since the calculations had been made on the basis of the anhydrous salt rather than $\text{Ca}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$.

per cent on a cloudy day. Additional light was provided every evening for about 4 hours by a series of 150 watt Mazda lamps suspended 3 feet from the growing tips of the plants. These lamps were controlled by an automatic time switch. The temperature of the house was maintained as nearly as possible at 25° C., and a relatively high humidity was maintained by frequent watering down of the concrete floor.

SAMPLING, ANALYSIS, AND EXPRESSION OF DATA.—After harvesting, the midribs were removed from the leaf blades. The latter were then placed in a hot air oven for 2 hours at 80° C. All analytical data were expressed upon this dry material as a basis.

Analyses for nicotine were made according to the silicotungstic

acid precipitation method after distillation from alkaline solution with steam (2).

In the present study the data were expressed as percentage of dry weight and as absolute amount per plant. Emphasis was given the percentage method, however, in view of the fact that a knowledge of the quality of tissue formed under the influence of varying types of nitrogen nutrition represented essentially the fundamental objective in this investigation.

INFLUENCE OF AMMONIUM NUTRITION UPON NICOTINE FORMATION IN LEAVES OF MATURING TURKISH TOBACCO PLANTS

A comparison of the effects of ammonium, urea, and nitrate nitrogen upon nicotine formation in maturing Turkish tobacco plants constituted the first phase of this study. Two concentrations of ammonium nitrogen were employed. One was molecularly equivalent to the nitrate and urea solutions; the second was equivalent to one-half the molecular concentration of these solutions, and was included to approximate a more ideal nutrient medium under the existing conditions of restricted carbohydrate synthesis than would theoretically be afforded by the more concentrated solution of ammonium salts. A brief characterization of the experimental procedure and results follows.

On November 14, approximately 5 weeks after initiation of the cultures, four groups of eight plants each were harvested and prepared for analysis. At that time, those plants supplied with nitrate (hereafter referred to for convenience as nitrate plants) were considerably taller than the plants of the remaining groups, and their leaves were a lighter green color. Likewise the dimensions and the dry weights of the nitrate plants greatly exceeded the corresponding values for the plants of all other groups.

No significant difference was observed, either in development or in dry weight, between the plants cultured in the two ammonium series (hereafter referred to as dilute ammonium plants and concentrated ammonium plants respectively). Compared with the nitrate plants, however, both groups of ammonium plants were less

well developed and were lower in dry weight. The concentrated ammonium plants possessed characteristically rugose, glossy, dark green leaves. The plants grown with urea nitrogen (designated hereafter as urea plants) were the smallest in all dimensions and the lowest in dry weight. The leaf color contrasted sharply with that of the ammonium plants in being a light yellow green. Data for this experiment are given in table 2.

The relative quantities of nicotine per unit of leaf tissue were identical in the nitrate and concentrated ammonium plants and in

TABLE 2
EFFECTS OF FORM OF NITROGEN ON NICOTINE CONTENT OF
TURKISH TOBACCO LEAVES

FORM OF NITROGEN	DRY WEIGHT OF LEAVES PER PLANT (GM.)	NICOTINE PERCENTAGE	TOTAL NICO- TINE PER PLANT (MG.)
Nitrate.....	5.83	1.26	73
Ammonium (concentrated)...	3.35	1.28	43
Ammonium (dilute).....	3.33	1.05	35
Urea.....	2.23	1.04	23

the dilute ammonium plants and urea plants respectively. The value for the first two was approximately 20 per cent above that for the latter two groups.

A number of apparently significant observations arise from the data just outlined. In the first place, the proportion of nicotine per unit of dry leaf tissue was the same for both the nitrate and the concentrated ammonium plants. Such a result was not in accord with the findings of BEAUMONT *et al.* (4), who reported that ammonium nitrogen nutrition definitely increased nicotine content per unit of mass. In the present case, nicotine concentration in the leaves seemed to be correlated with quantity rather than with quality of nitrogen supplied to the roots.

In the second place, the nicotine content of the leaves of the dilute ammonium plants was 15 per cent below that of the leaves of the concentrated ammonium plants, although their respective dry weights were practically identical. The only apparent conclusion

seems to be that the dilute ammonium solution represented a concentration of nitrogen which quantitatively limited nicotine formation.

On the other hand, leaves of the urea plants, although attaining only about two-thirds the dry weight of the dilute ammonium plants, yet possessed the same percentage content of nicotine. It was evident throughout the course of the experiment that urea was inferior to either ammonium or nitrate as a nitrogen source. This result is in contrast to the reported findings of others (1, 4) in which urea employed in water cultures and in field experiments gave results intermediate between ammonium and nitrate, or even superior to both, as regards plant growth.

INFLUENCE OF AMMONIUM NUTRITION UPON NICOTINE
SYNTHESIS IN LEAVES OF IMMATURE HAVANA SEED
TOBACCO PLANTS

A second phase of the study was concerned with the effects of nitrate and ammonium nutrition upon the nicotine content of immature tobacco plants of the Havana Seed variety under conditions unfavorable to the elaboration of carbohydrates. This experiment was begun on November 19, 1937, at which time the plants were about 10 cm. in height and bore from three to four leaves each. The methods employed were exactly the same as for the previous experiment, except that the nitrate solutions were left at their own pH of 6.4. The ammonium solution used was the concentrated solution of the previous experiment. The plants were placed on the north bench in the greenhouse where they were exposed to reduced light and temperatures. As a result, growth was very poor, and at the end of 23 days the plants had put forth only three or four new leaves each. The plants were harvested and dried at this point.

In contrast to the previous experiment, a sizable increase in the percentage of nicotine was found in the ammonium leaf tissues. Furthermore, the absolute nicotine content of the ammonium leaf tissues showed the same quantitative increase when based on the number of grams per plant. The nitrate plants developed the typical light green leaf color already mentioned in connection with the previous experiment, while the ammonium plants possessed glossy, dark

green, rugose leaves with a much greater water content. Table 3 gives the dry weight and nicotine values for this experiment.

The increased percentage of nicotine in the ammonium plants is perhaps not out of harmony with the results of other studies, since it has been rather conclusively shown that nicotine synthesis is influenced to some extent by the level of nitrogen nutrition (7), also that ammonium nitrogen is assimilated by plants in the earlier stages of development much more readily than nitrate nitrogen (8). If there is any relationship between these observations and the re-

TABLE 3
EFFECTS OF AMMONIUM AND NITRATE NITROGEN ON NICOTINE
CONTENT OF HAVANA SEED TOBACCO LEAVES

FORM OF NITROGEN	DRY WEIGHT OF LEAVES PER PLANT (GM.)	NICOTINE PERCENTAGE	TOTAL NICO- TINE PER PLANT (MG.)
Nitrate.....	0.69	0.92	6
Ammonium.....	0.74	1.33	10

sults obtained in the present study it probably lies in the early stimulation of the nicotine synthetic process, due to an increased assimilation of nitrogen in the ammonium plants rather than to a sustained higher rate of nicotine formation from the beginning of the experiment. In any case, the main conclusion seems to be that in the early stages of growth, even under conditions essentially unfavorable for carbohydrate synthesis, the formation of nicotine proceeds at a more rapid rate in tobacco leaves under the influence of ammonium nitrogen than under the influence of nitrate nitrogen.

INFLUENCE OF AMMONIUM AND NITRATE NUTRITION AND SOIL CULTURE UPON NICOTINE FORMATION IN CONNECTICUT BROADLEAF TOBACCO DURING THE PERIOD OF MOST ACTIVE GROWTH

The difference in results obtained between the last two experiments suggested the desirability of still further investigation designed to follow the changes in nicotine content throughout the period of most active growth of the plant under conditions of ammonium and of nitrate nutrition and of limited light. It was hoped

to learn whether or not there is an initial rapid synthesis of nicotine in ammonium culture followed by a gradual decline in rate of formation during the later stages of growth. It was further considered desirable to include as controls a similar set of plants grown in soil in order that the differences between the nitrate and ammonium cultures might be more fully evaluated. The plants were placed under experimentation when 70 days old and were harvested in four crops, beginning at the eighty-eighth and extending to the one-hundred-twelfth days. The last sample was collected on March 28.

TABLE 4

EFFECTS OF AMMONIUM, NITRATE, AND SOIL CULTURE UPON NICOTINE CONTENT OF CONNECTICUT BROADLEAF NO. 38 TOBACCO LEAVES

DAYS FROM SEED	DRY WEIGHT OF LEAVES PER PLANT (GM.)			NICOTINE PERCENTAGE			TOTAL NICOTINE PER PLANT (MG.)		
	NO ₃	NH ₄	SOIL	NO ₃	NH ₄	SOIL	NO ₃	NH ₄	SOIL
88.	1 3	0 9	1.4	1.05	1.39	2 03	14	13	28
99.	3 4	3.3	3.1	1 04	0 98	1 47	35	32	46
108.	7 6	5.4	7.9	0 90	0.90	1.28	68	49	101
112.	11.5	7 0	9.4	0 88	0 89	1.50	101	62	141

Twenty-four plants of the Connecticut Broadleaf no. 38 variety were employed, twelve each in nitrate and ammonium culture. The conditions of lighting, the composition and rate of flow of the nutrient solutions, and the temperature and humidity of the greenhouse were as nearly as possible adjusted to duplicate the conditions employed in the first of these experiments. At the time of transplanting the seedlings to sand, a number of uniform individuals were also transferred to 8 inch pots of soil, where they were cultured as described earlier in this paper. The sand employed was not the white quartz sand previously used but was a yellow sea sand obtained from Cow Bay, New York, which had an average porosity equal to that of the white quartz sand, but which had in addition a much wider range of particle size. Measurement of both sands with standard mesh sieves indicated a very uniform porosity of 42 per cent for the white sand and an average porosity of 42 per cent for the yellow

sand, with a range for the latter between 40 and 44 per cent. This sand was thoroughly washed with running water until free from fine debris before using.

Growth in the ammonium series followed a very regular course, in contrast with the more normal sigmoid type of growth curve of the soil and nitrate plants. It is likely that the final flattening off of the curve (fig. 1) for the soil plants was due to root binding within

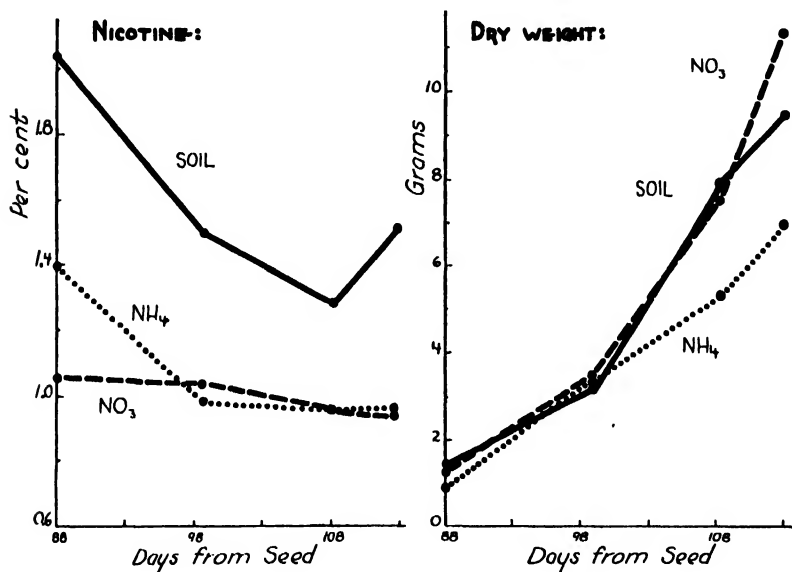


FIG. 1.—Effects of culture in sand with ammonium and nitrate nitrogen and of soil culture upon dry weight and nicotine content of Connecticut Broadleaf tobacco leaves.

the clay pots, since growth of both soil and nitrate plants had been very similar up to that point.

The nicotine content of the leaves expressed as percentage of dry weight presented some interesting variations (fig. 1). In the first place, the observations of earlier experiments seem to have been completely upheld in regard both to the initial rise in nicotine content under the influence of ammonium nitrogen and to the final equivalence of the nicotine values for both ammonium and nitrate plants. The data bring out further details of these relationships, however, which have not been made evident in the preceding experi-

ments. In both cases those plants in the sand cultures possessed a higher percentage of nicotine in their leaf tissues at the time of first sampling than they did at later samplings, and the decrease in percentage values was fairly uniform from the first down to the last sample. Furthermore, inspection of each corresponding pair of decreasing values reveals that the relative nicotine contents of ammonium and nitrate leaves were very nearly the same in each sample after the first. Nicotine in the leaves of the soil plants had attained a concentration considerably in excess of that in the leaves of the ammonium and nitrate plants at the time of first sampling. Subsequently the nicotine content of the soil plants decreased irregularly until, at the time of last sampling, it had reached a value nearly 1.7 times that of the final values for the ammonium and nitrate plants. In the absence of further data, it may be suggested that the increased nicotine content of the leaves of the plants in soil was associated with a hydration factor (6).

Summary

1. The influence of ammonium, nitrate, and urea nitrogen upon nicotine synthesis in tobacco leaves has been investigated under conditions unfavorable for optimum carbohydrate formation.

2. Ammonium nitrogen compared with nitrate seemed definitely to have increased the relative nicotine content of tobacco leaves in the earlier stages of growth. This influence was soon lost, under the conditions of the experiments, and the ammonium-cultured plants, although lower in dry weight and showing definite indications of ammonium toxicity, subsequently possessed nicotine concentrations indistinguishable from those attained by the nitrate-cultured plants of the same age.

3. No essential difference was observed between the effects of "concentrated" and "dilute" ammonium nitrogen upon plant growth and dry weight. However, the nicotine content responded to the increased nitrogen supply of the concentrated ammonium solutions by assuming somewhat higher proportions in the leaves.

4. Growth of plants supplied only with urea nitrogen was inferior to the growth of plants cultured in ammonium solutions, either at concentrations of ammonium equivalent to or half that of the urea.

The influence of urea on nicotine percentage was equivalent to the influence of one-half the same molecular concentration of ammonium salts.

5. Solution cultures, regardless of the form of nitrogen supplied, induced a lower concentration of nicotine in the leaves than did soil cultures even though the dry weights of the leaves were the same. Such an action may have been associated either with differences in the moisture content and aeration of the culture medium or with qualitative or quantitative differences in the salt composition of the culture solutions.

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HISTOLOGICAL RESPONSES OF CABBAGE PLANTS GROWN AT DIFFERENT LEVELS OF NITROGEN NUTRITION TO INDOLE(3)ACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 495

ETHEL GOLDBERG

(WITH TEN FIGURES)

Introduction

Considerable histological work has recently been done on the responses of various plants to applications of growth promoting substances. FISCHNICH (3) worked with *Coleus*; KRAUS, BROWN, and HAMNER (9), HAMNER and KRAUS (7), and LINK, WILCOX, and LINK (11) with bean; BORTHWICK, HAMNER, and PARKER (2) with tomato; HARRISON (8) with *Iresine*; and HAMNER (6) with *Mirabilis*. All reported great stimulation of various tissues, resulting in the production of callus and root primordia. GREENLEAF (5) noted the production of shoots from the cut surface of *Nicotiana* treated with indole(3)acetic acid, but reported no histological work. BEAL (1) made a detailed study of bud development from leaf axils in *Lilium harrisii* induced by applications of indole(3)acetic acid to the cut surface of the decapitated stem.

Cabbage was found to be very responsive to indole(3)acetic acid, and to produce shoots from the decapitated surface of a large proportion of treated seedling stems (4). It was also noted that the reaction of the plants, at least in rate, to applications of this substance was largely dependent upon the nutritive state of the plant. These observations were made the basis of the anatomical study reported here.

Material and methods

About 2000 young cabbage plants of the Wisconsin All Seasons variety were used throughout the experiments. All were grown under ordinary greenhouse conditions of light and humidity. Some were grown in soil, while others were grown in sand in the Chicago

soil-temperature nutrient tanks, which maintain the root temperature at 20° C. with a variation of only 1° in either direction. There was an average diurnal range in greenhouse temperature of 13° C. (24° to 37° C.). The plants in sand were watered each day with either Shive's R_4S_5 nutrient solution, or the same solution in which the calcium nitrate had been replaced by calcium chloride.

A 3 per cent mixture of indole(3)acetic acid (Merck) in lanolin was used for all applications. Material for histological work was collected at intervals of 6 hours for the first 72 hours; every 12 hours for the next 144 hours; and every 24 hours thereafter until 504 hours had elapsed. It was fixed in Navashin's solution and imbedded by the butyl alcohol-paraffin method. Sections were cut at 12 μ and stained with triple stain.

Experiments and results

SEEDLINGS GROWN IN SOIL

DECAPITATED FIRST INTERNODES.---Seedlings 20 days old grown in ordinary greenhouse soil were cut squarely across near the top of the first internode. The cut surface was then smeared with the indole(3)acetic acid mixture. Controls were smeared either with lanolin or were left with no treatment other than decapitation.

At the time of treatment the stems had just begun to undergo secondary thickening (fig. 2A). A well defined epidermis and cortex have developed, the outermost cells of which contain abundant chloroplasts. The endodermis can be distinguished from other cortical cells, although with difficulty, by the denser cytoplasm and somewhat more prominent nuclei. Sieve tube and companion cells of the primary phloem are mature, and one or two rows of pericyclic cells still showing protoplasmic content cap each phloem group. The cambium is just beginning its activity, with not more than two rows of as yet undifferentiated cells on either side. Prominent xylem rays are apparent in some of the larger bundles. The pith is large in extent, with comparatively narrow medullary rays passing between the bundles.

Within 18 hours after treatment, cell enlargement in the pith is apparent. By the end of 24 hours, the cortical cells also show an

enlargement. After 54 hours the size of these cells has almost doubled, and epidermal cells have also enlarged to keep pace. This is the maximum enlargement of individual cells of the pith; occasionally some cortical cells in later stages may almost triple their size.

By the end of 30 hours an increase in the activity of the cambium and a delay in maturation of its derivatives are apparent. The endodermis is much more easily identified, owing to the increased density of the cytoplasm and prominence of its nuclei. Many of the phloem parenchyma cells enlarge, and these, together with primary xylem parenchyma, rays, and a few pith cells adjacent to the protoxylem points, show dense cytoplasm and very prominent nuclei. An increased density of protoplasm is also apparent in some of the pericyclic fibers, but it is doubtful whether they play any part in further activity within the stem.

First divisions in the endodermis appear in 42 hours. These may be in any plane, and within 48 hours some cells have undergone division into three, while others are multinucleate. The outermost cells of the medullary and bundle rays divide rapidly and their derivatives increase in size until they push aside the inactive pericyclic cells. There is some division in the primary phloem and the cortex.

Within 60 hours the sites for development of later root primordia have been determined owing to the extremely rapid division of the ray cells and the somewhat less rapid, but nevertheless marked, division of the phloem parenchyma. Certain of the endodermal cells have divided into as many as four (fig. 2*B*), which form a cap over the dividing ray cells. Pericyclic cells are dispersed and in some cases the phloem cells thus come to lie next to the endodermis. The primary phloem cells become crushed.

At the end of 144 hours the histogens of the root primordia have become organized, mainly from ray tissue flanked by phloem derivatives. A cap of endodermis 3-6 layers of cells wide is pushed outward by the growth of the root, which soon pushes through the cortex and epidermis (fig. 3*A*).

Numerous xylem elements mature, some of which differentiate back to abut against the secondary xylem cells of the stem. The xylem parenchyma proliferates to the extent that the original pro-

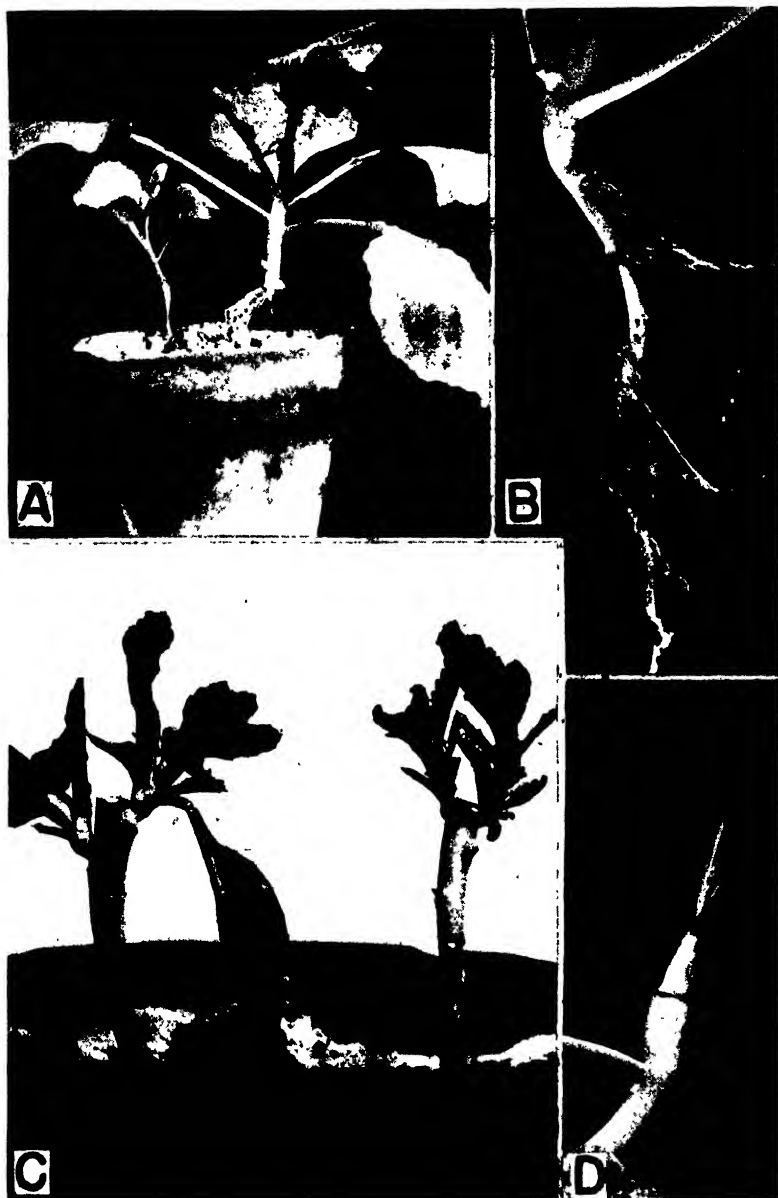


FIG. 1.—A: +N and -N control plants showing gross differences 1 week after treatment of other plants of series. B: +N plant 3 weeks after placing small daub of lanolin mixture on unwounded internode and covering with sand. C: decapitated plants 3 weeks after wounding; plant on left untreated; one on right smeared with lanolin mixture. D: unwounded internode 4 days after ringing with fine thread of lanolin mixture. Ink circle drawn on site of application just before photographing.

toxylem points are dispersed. The parenchyma of the proximal parts of the rays, together with the adjacent pith and the pith adjoining the protoxylem points, proliferate greatly, producing a confused array of vascular strands, disorganized tracheids, and highly meristematic areas which may be circular, semicircular, or straight, and oriented in any direction. The vascular strands produce mainly xylem elements with a few irregularly placed phloem cells. Large mounds of callus are usually produced, mainly by the phloem. Root primordia in large numbers are produced only in the region of the original stem, below the callus. They are usually fasciated.

The vascular tissue maturing in the callus at first differentiates downward and connects with the vascular tissue of the root primordia. Usually only disjointed groups of vascular tissue are apparent later. These may be large, or so small as to consist of only one tracheid. The large groups may have very well developed cambiums which run in any direction and then abruptly end. The whole presents a picture of extreme confusion.

About one-third of the plants investigated produced shoots as well as roots, although in such cases the number of roots was greatly reduced (figs. 3*B*, 4, 5, 6). Shoots arise either laterally from tissues outside the vascular bundles which have been stimulated by the indoleacetic acid or from the callus over the surface of the cut end. No plants were noted which produced both lateral and apical shoots. Several shoots of either kind were produced by one plant, as many as ten having been counted on an individual stem. There appears to be no functional difference between shoots produced in either position, the only difference being in point of origin. The lateral shoots orient themselves in an upright position; and since they are located so close to the apical end, after some little growth they cannot be told apart from the others except with great difficulty.

Shoots arising laterally (fig. 4) are initiated by rapid cell divisions, either in the central or innermost cell layers of the cortex or in the rays. Since they originate in the region of the stem which produces adventitious roots, in the earliest stages they cannot be distinguished from young roots except that they do not have a cap of endodermis. In their outward growth, tissues in their path are crushed, and when about to emerge through the epidermis a terminal meristem be-

comes organized. Vascular tissue differentiates back until a connection is made with that of the main stem. Soon a smooth juncture of cambiums of new and old stems comes about and the shoot continues its growth as would the original stem tip.

Shoots arising terminally involve a greater number of tissues. Most of them were found to arise from external callus produced by either the pith or the phloem (figs. 3*B*, 5, 6), and never a combination of the two. Cortex, xylem, cambium, and endodermis may also be involved with either pith or phloem to a small extent. Here, as in the lateral shoots, a complete tieup of new and old vascular tissues is effected, either directly as in figure 6, or indirectly as in figure 7. The meristematic areas seen clearly in the pith of the latter figures are connected directly with a large shoot above that level, and indirectly by means of vascular strands to the organized vascular system of the old stem below that level.

Control plants produce no mounds of callus tissue at the cut surface. On the contrary, more of the layers of pith cells beneath the cut die than do those over the other tissues, thus causing a sunken appearance in the center. Cell divisions in a horizontal plane occur, so that a weak phellogen is produced across the cut surface, which remains about as flat as at the time of decapitation.

UNWOUNDED AND WOUNDED FIRST INTERNODES.—A small daub of the 3 per cent mixture was placed on the first internodes just below the petiole of the first leaf of 28-day-old seedlings grown in soil. Care was taken not to injure the stem. Gross reactions of unwounded plants were somewhat faster than those of decapitated ones. Reaction occurred almost entirely below the point of application, very slightly above, and was not apparent laterally to any great extent. Under the application of the paste an elongated, narrow lesion appeared, which became sunken and brown, extending downward through the entire internode. Through this the more or less longitudinally regular rows of adventitious root primordia protruded by the end of 9 days. These did not elongate further under the conditions in which they were grown.

The fourth internodes of 45-day-old seedlings were encircled with an extremely fine band of the lanolin mixture. Here again the reac-

tion was limited almost completely to those portions of the stem below the site of application (fig. 1*D*). A definite swelling of the entire internode, which was first apparent within 24 hours, was soon extended downward through several internodes. Within 48 hours after treatment a pronounced whitening of the stem throughout the swollen portion occurred, followed within about a week by the production of root primordia.

Except for two features, the early stages of histological reactions were similar to those of decapitated plants. The activation of all tissues except the pith and the centripetal ends of the rays was the same as in that of decapitated plants. These two tissues, however, showed no response to the application (fig. 8*B*), and could not be distinguished from those of untreated plants. The second feature was the lack of protruding callus. If the treated part of the stem was not placed in a moist chamber, the rows of root primordia eventually became buried by a smooth mound of callus, and the lesion apparently healed over.

SEEDLINGS GROWN IN SAND: RESPONSES IN RELATION TO NITROGEN DEFICIENCY

Seedlings 40 days old which had been grown in sand and supplied with complete nutrient solution were separated into two groups, one of which was continued on the complete nutrient while the other was given the nutrient solution which lacked nitrates. Ten days later, which was at the time of treatment, the $-N$ plants tested negative to diphenylamine. They were lighter green in color, somewhat smaller in size, and a little woodier than the $+N$ ones. These differences became much more pronounced with the progress of the experiment, as can be seen by figure 1*A*, which shows a control plant of each group.

DECAPITATED PLANTS.—The $+N$ plants were decapitated between the seventh and eighth nodes, while the $-N$ ones, owing to their shorter height, were decapitated between the sixth and seventh nodes. This was as close to the top of the plant as it was possible to cut and get a smooth internode.

The general histological response pattern under both conditions of nutrition was the same, although differences were apparent in the growth rate and somewhat in the type of reaction of the different tissues involved.

At the time of treatment the average stem diameter of the $-N$ plants was about 15 per cent smaller than that of the $+N$ plants. The cortex of the latter was relatively wider and its pith was relatively very much larger than that of the former, the differences being ascribable to number of cells and not to cell size. Cell walls of the $-N$ plants, especially those of the pericyclic fibers, were heavier, the relative amount of vascular tissue was much greater, and the cambial activity was less than that of the $+N$ ones (fig. 8C, D).

By the end of 48 hours (fig. 9A) considerable cell divisions in the rays, primary phloem parenchyma, and endodermis, and some in the pith have occurred in the $+N$ plants, while in the others (fig. 9B) the only apparent response is a prominence of nuclei in the endodermis and rays.

Within 90 hours the root primordia of the $+N$ plants (fig. 9C) have been almost completely organized, while the cortex, endodermis, phloem, rays, and pith cells are all actively dividing. Mounds of callus tissue are appearing over the top of the cut surface. The tissues are about equally activated along all radii. The $-N$ plants of this age (fig. 9D) are not quite so far along in development as were the $+N$ plants in 48 hours, and their vascular cylinders are very unequally activated, large portions remaining unresponsive.

By 132 hours the ring of root primordia of the $+N$ plants (fig. 10B) has pushed out so far as completely to disrupt the cortex and epidermis. An inner ring of root primordia, derived from pith cells especially opposite the pith rays, are growing inward. This is not apparent in all the plants studied, however. Many circular vascular strands are forming in the rays between the protoxylem points (fig. 8A). On the other hand, root primordia of the $-N$ plants are not yet fully organized. Activity in the pith and cortex is much less pronounced, and divisions are confined mostly to rays, phloem, and endodermis (fig. 10A).

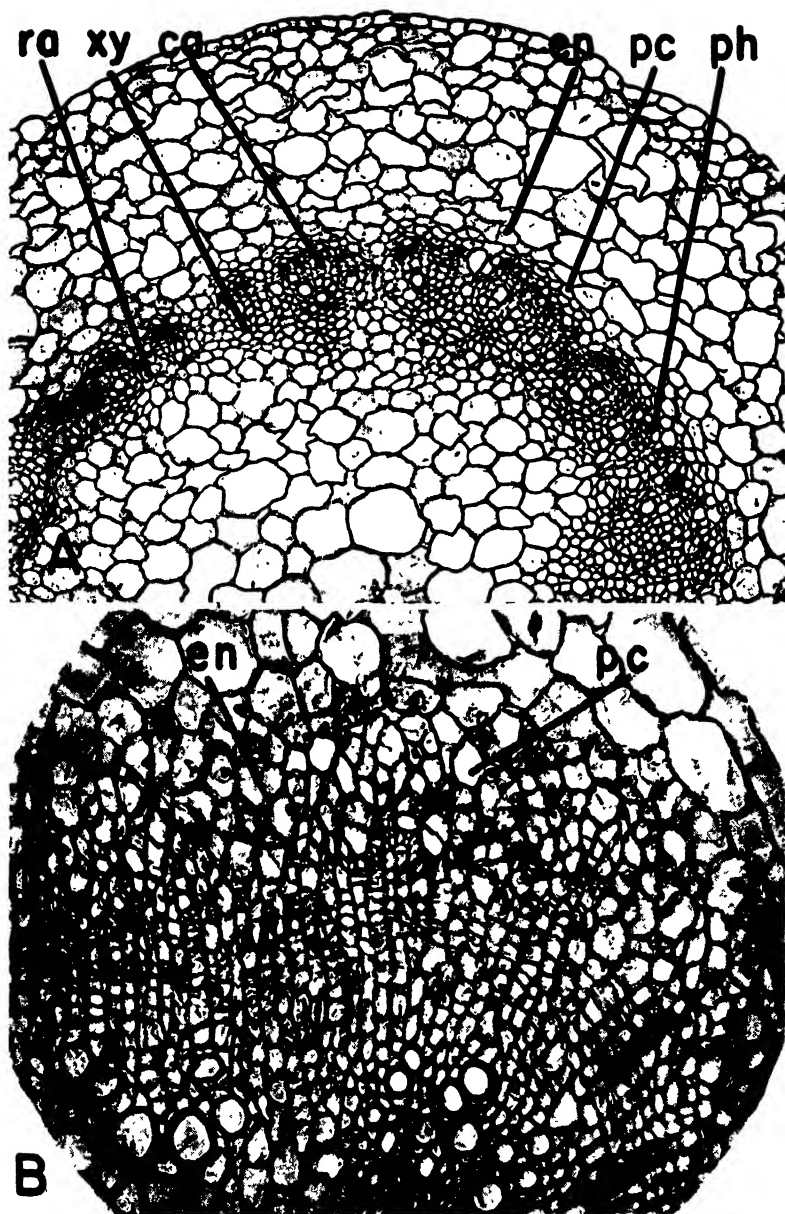


FIG. 2.—Decapitated first internodes of young plants in soil smeared with lanolin mixture. *A*: at time of treatment (*ra*, ray; *xy*, xylem; *ca*, cambium; *en*, endodermis; *pc*, pericycle; *ph*, phloem). *B*: 60 hours after treatment. Note activity in cambium, rays, endodermis, and phloem; and inactivity in pericycle.

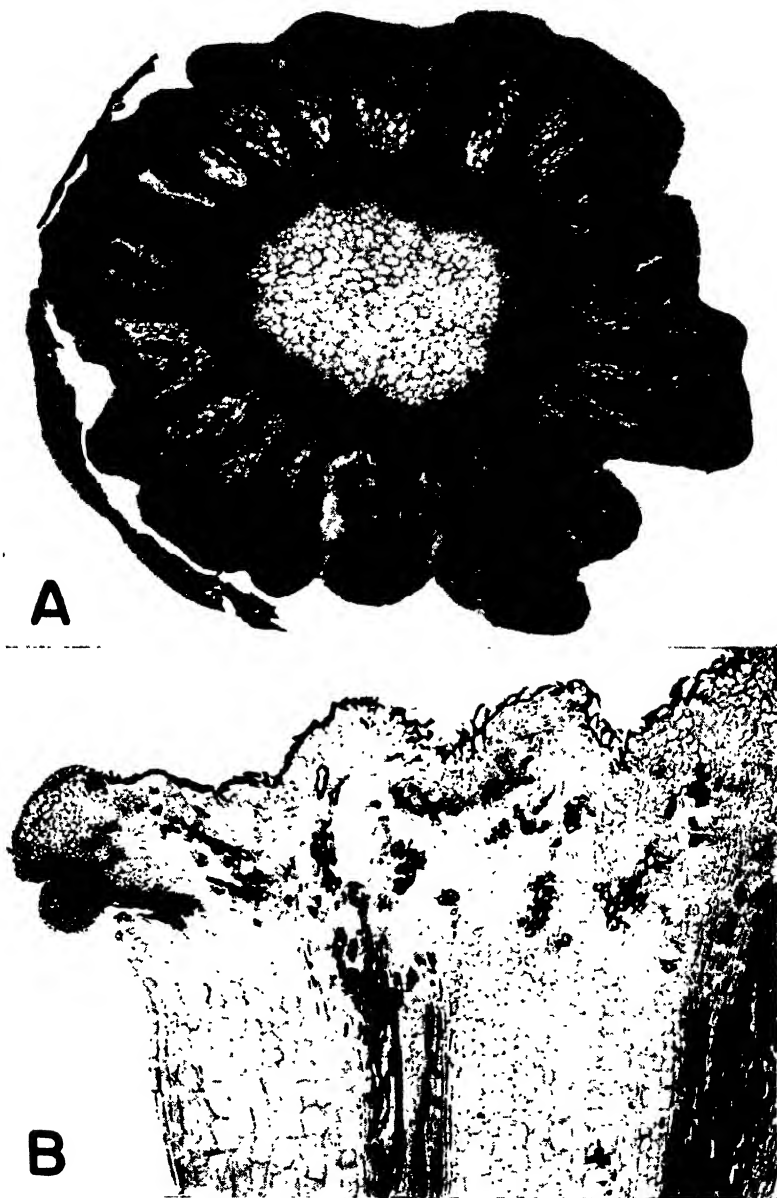


FIG. 3.—*A*: 336 hours after treatment. Root primordia pushed out through epidermis and cortex. Note activity in periphery of pith. *B*: decapitated and treated first internode of plants grown in soil, 408 hours after treatment. Terminally produced shoot involving mostly cortex, also endodermis and phloem to some extent.

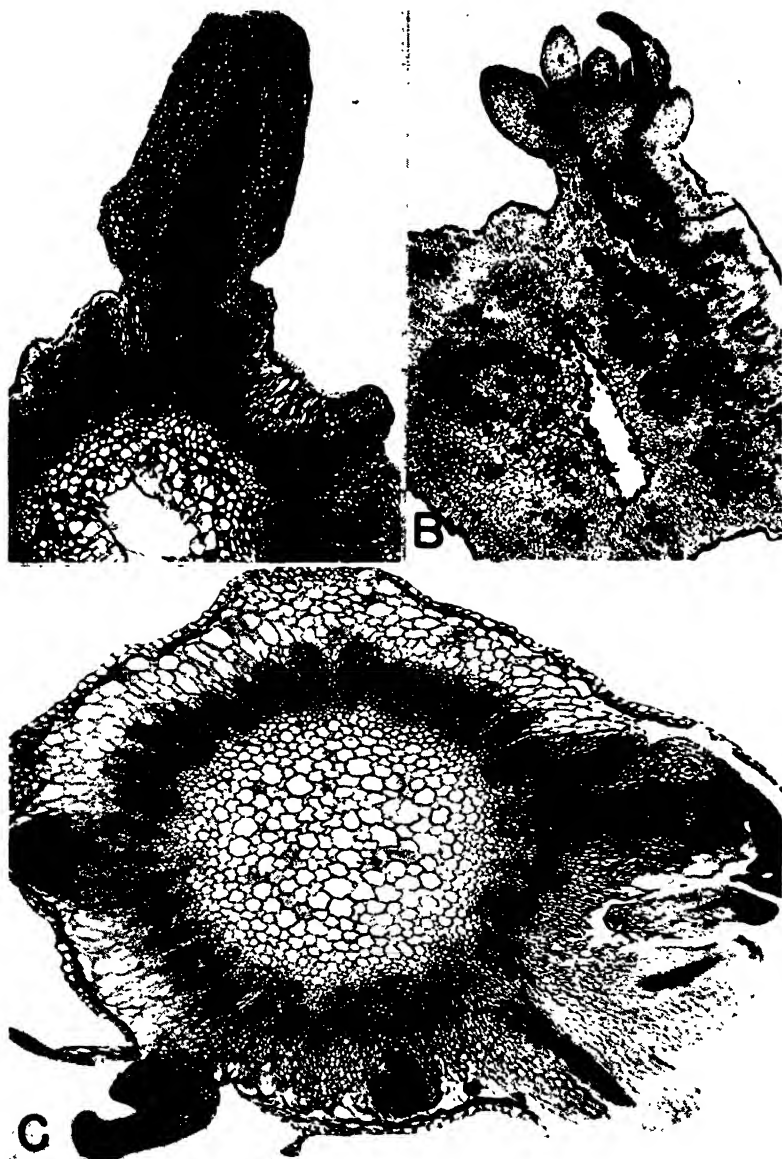


FIG. 4.—Decapitated and treated first internodes of plants grown in soil. *A*: 396 hours after treatment. Shoot produced laterally from cortex has established complete vascular connections with original stem. *B*: 408 hours after treatment. Shoot arising from callus developed over cortex has established vascular connections with vascular strands which in turn connect with vascular system of main stem. Note lack of callus developed over central part of pith and irregularity of maturation of vascular elements within callus. *C*: 396 hours after treatment. Two older shoots developed from cortex; two younger ones arising in same tissue.

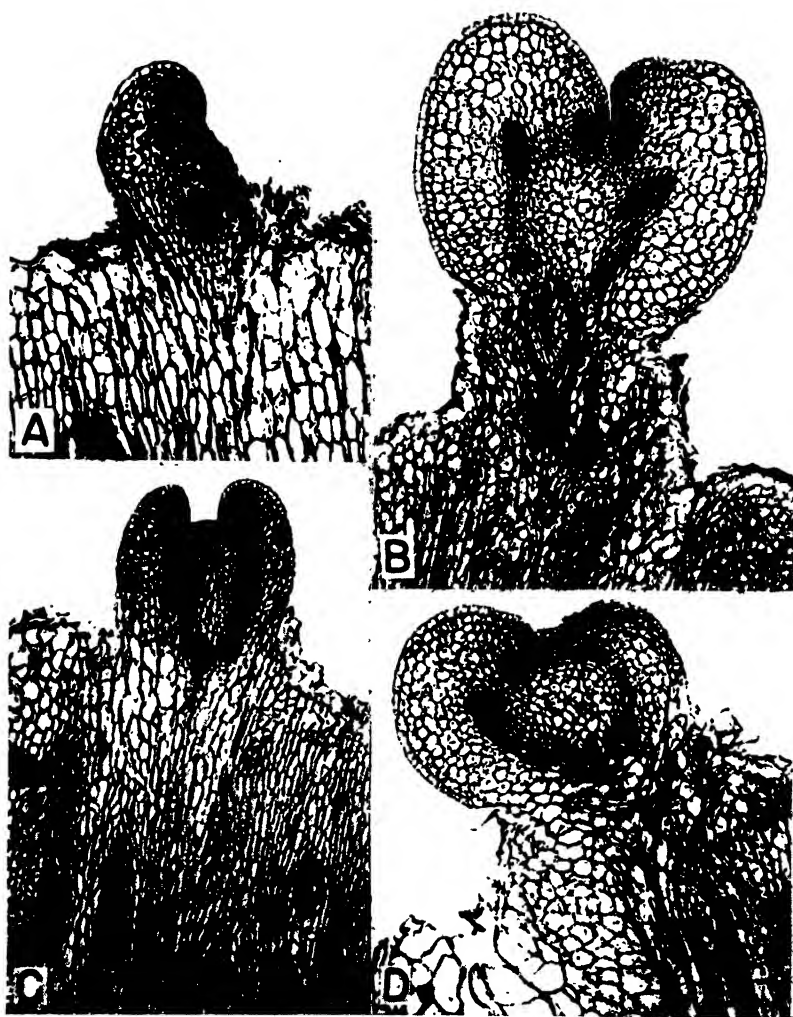


FIG. 5.—*A, B, C, D*: decapitated and treated first internodes of plants grown in soil. Shoots originating from callus produced over phloem.



FIG. 6.—Decapitated first internodes of plants grown in soil and smeared with 3 per cent lanolin mixture. *A*: 288 hours after treatment; shoot produced from callus mostly over pith, with some involvement of xylem. *B*: 396 hours after treatment; shoot arising from phloem.

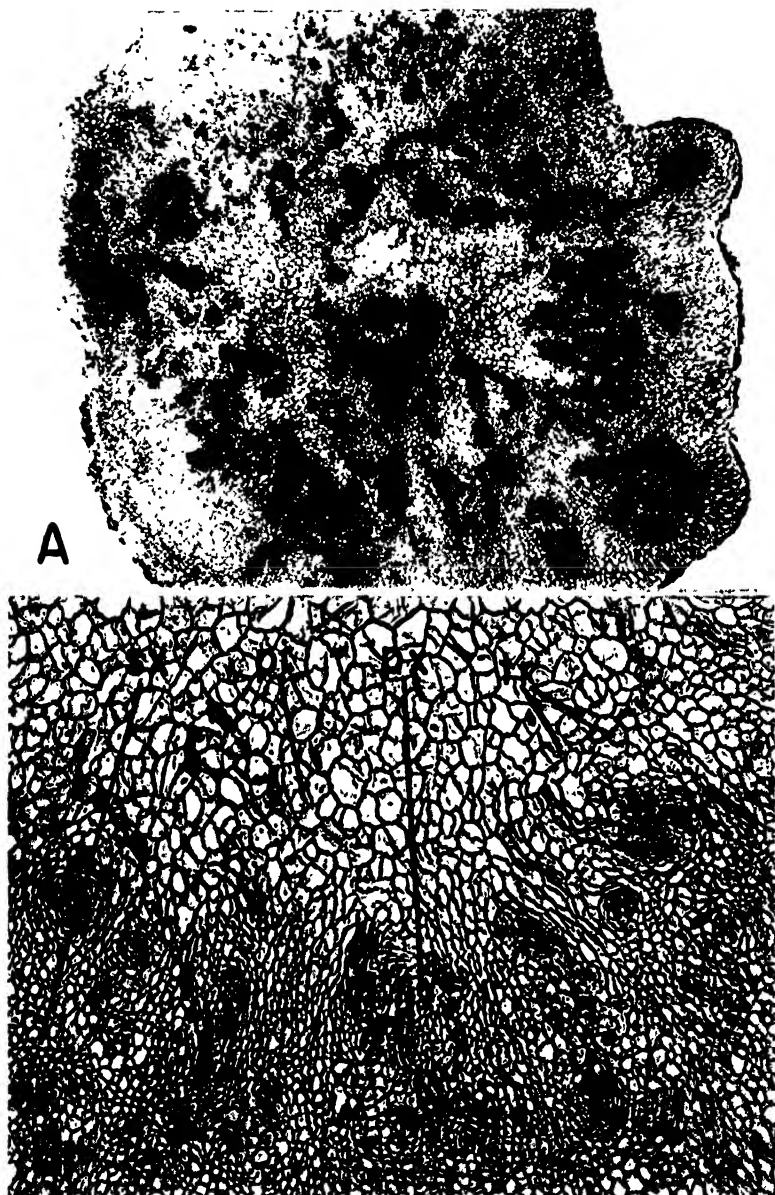


FIG. 7.—*A*: similar stem, 372 hours after treatment. Note great activity in pith, connecting above with a shoot, below with main stem. *B*: stem similar to that of *A*, one portion enlarged. Note development of vascular strands, maturation of irregularly oriented tracheids, and great numbers of cell divisions in pith.

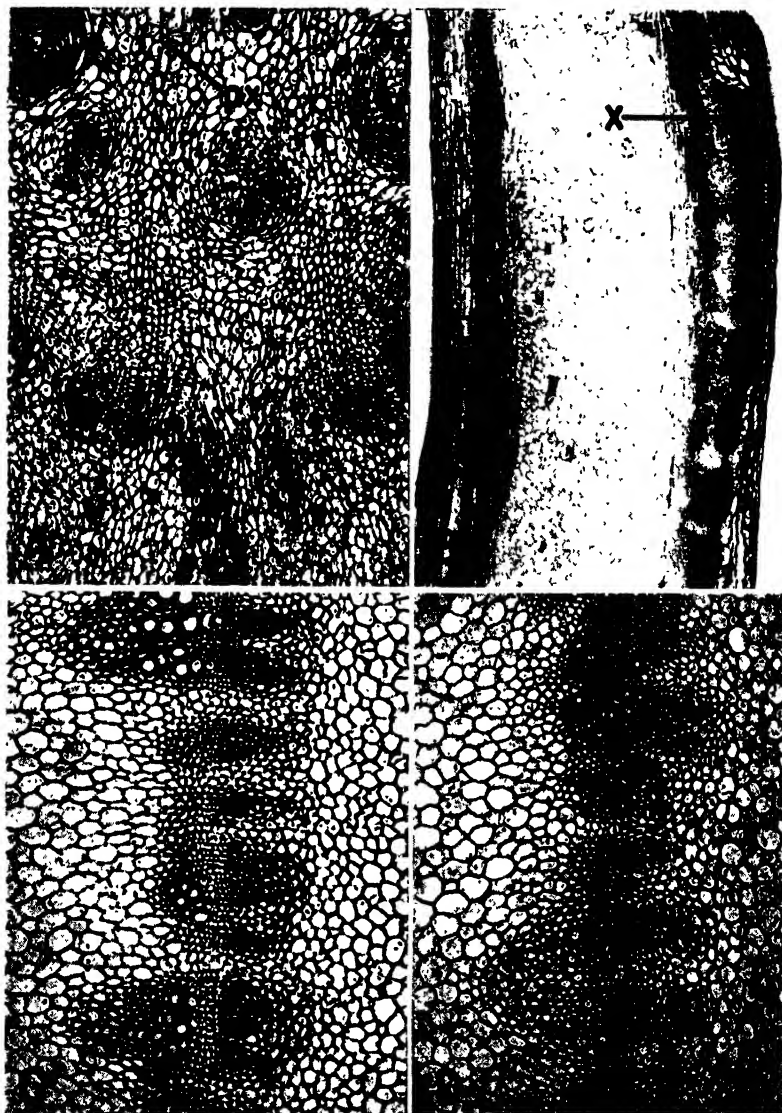


FIG. 8.—*A*: stem similar to that of fig. 7*A* and *B*, but at lower level and still further enlarged. *B*: unwounded first internode, treated by placing small daub of 3 per cent paste at *X*. Note most of activity below point of application and none on opposite side. *C*: +*N* plant, grown in sand, at time of treatment. *D*: −*N* plant, other conditions same as *C*. Diameter of entire stem of *C* actually more than twice that of *D*; therefore note lesser relative amount of vascular tissue in *C*, also its more active cambium, wider rays, and thinner walled pericyclic fibers than *D*.

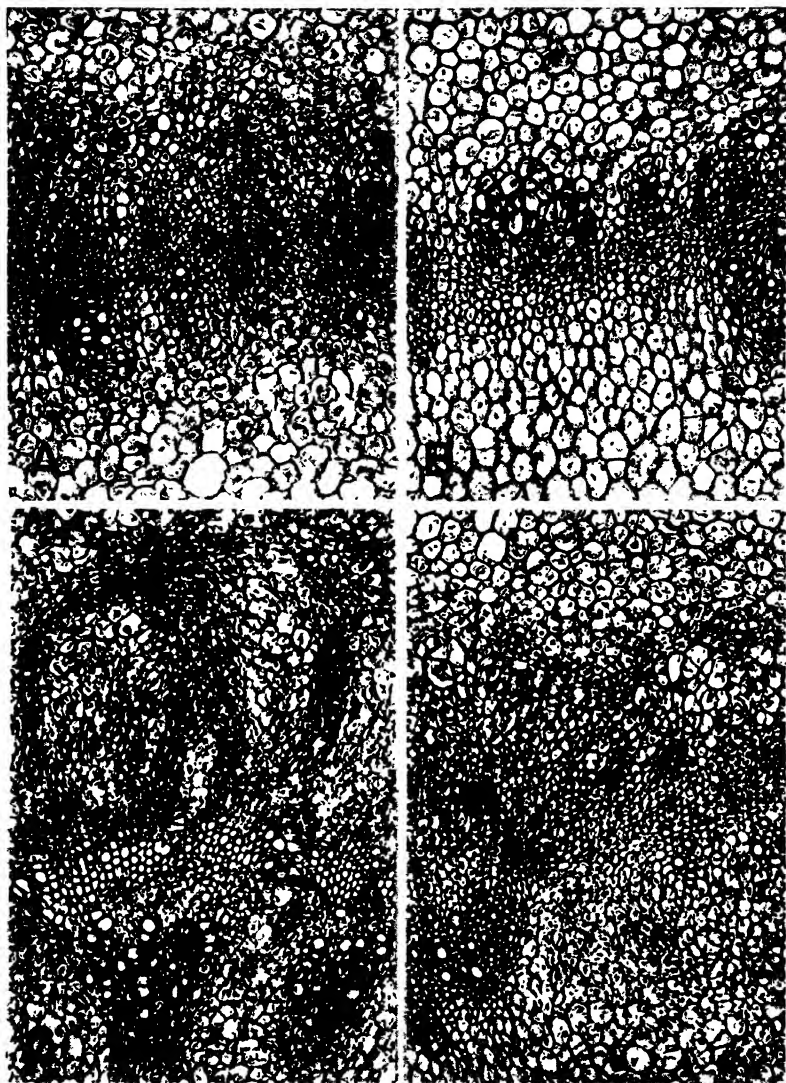


FIG. 9.—Plus N or -N plants decapitated near top of stem and smeared with 3 per cent lanolin mixture. *A*: +N, 48 hours after treatment. Great activity in rays, cambium, and phloem, some activity in endodermis and periphery of pith. *B*: -N, 48 hours after treatment. No change from time of treatment except prominence of nuclei in endodermis and rays. *C*: +N, 90 hours after treatment. Location of root primordia determined. Reactive tissues shown in *A* plus the cortex have proliferated extensively. *D*: -N, 90 hours after treatment. Large numbers of cell divisions in endodermis, rays, and cambium, some divisions in cortex and pith.

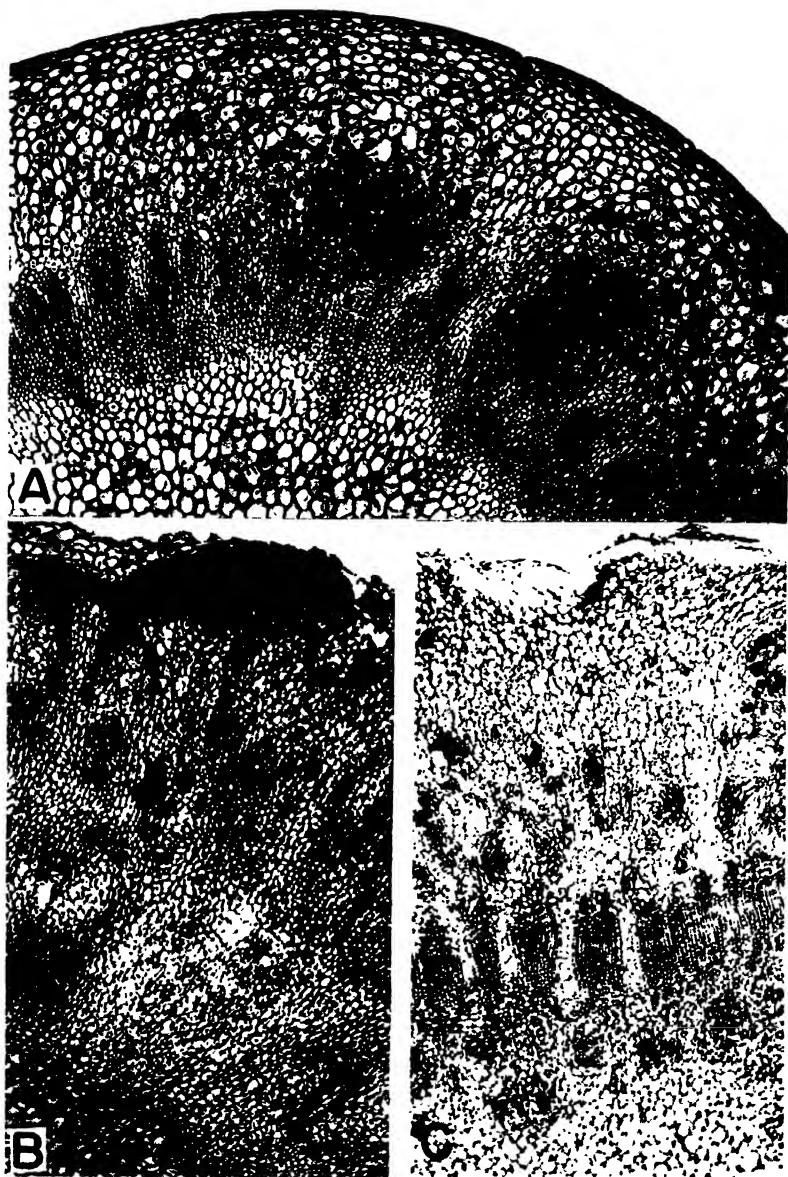


FIG. 10.—Plants similarly treated to those of fig. 9. *A*: -N, 132 hours after treatment. Root primordia just being organized. Note unequal activation of vascular cylinder and comparative inactivity of all tissues especially pith and cortex. *B*: +N, 132 hours after treatment. Note well developed root primordia, vascular strands, root primordia in pith, proliferation of xylem parenchyma which has pushed apart primary xylem elements. Epidermis and most of cortex gone. *C*: -N, 240 hours after treatment. Below level shown in *A*. Note scattered groups of fibers in cortex.

In final development (fig. 10C), the $-N$ plants produce very few, irregularly placed root primordia which rarely penetrate beyond the confines of the outer cortex and epidermis. Very little callus tissue is produced and this is less disorganized than the calluses of the $+N$ plants. The fewer meristematic patches are smaller in extent, tend to be tangential or radial rather than circular, are largely confined to the cortex and a short lateral extent in the pith; they are more regularly vascularized, producing complete vascular strands with phloem fibers, cambium, and xylem in the same relative relationship as in the original vascular cylinders.

Many patches of fibers are scattered throughout the cortex, where cortical cells have divided and their daughter cells have matured shortly thereafter. There is somewhat less tissue breakdown than is apparent in the $+N$ ones in which the phloem flanking the root primordia is disintegrating. Since the pith is not highly active, all of the innermost root primordia and many of the meristematic areas in the pith are lacking.

UNWOUNDED INTERNODES.—The unwounded internodes of $+N$ and $-N$ plants reacted relatively as did the decapitated ones, with no pith development occurring in either case. The root primordia of the $-N$ plants could rarely be induced to grow out, even when covered with moist sand. Figure 1B shows a $+N$ plant which had been covered by moist sand at the place of treatment so that the root primordia lengthened and became a functional part of the root system.

Discussion

The reaction of the cabbage plant to applications of the hormone is very similar to that of bean as described by KRAUS, BROWN, and HAMNER (9). Cabbage when untreated produces no mass of callus tissue over the cut surface. In this respect it is unlike the bean but similar to the tomato (2) and *Iresine* (8).

The epidermis and cortex of cabbage respond similarly to those of bean, tomato, *Iresine*, and *Mirabilis*. Endodermis of cabbage reacts in a manner similar to that of tomato and *Mirabilis*, being less active than that of bean and more so than that of *Iresine*. Pericycle

of cabbage is inactive, resembling bean and tomato in this respect. The vascular tissues differ in their responsiveness from those of *Mirabilis*, the latter being relatively unresponsive while those of the other three are sensitive to applications.

Adventitious root production in cabbage closely parallels that of bean, in that it involves mainly the rays plus proliferated phloem, and does not involve pericycle as do *Iresine* and *Mirabilis*. Cabbage pith is very active and produces large numbers of irregular vascular strands which in later stages extend into the center of the stem; this reaction is similar to that of bean, and differs from *Mirabilis* in that its activity is initiated very early.

Inhibition or at least retardation of bud growth has been reported in cases where decapitated plants have been treated with growth promoting materials. In cabbage, as in *Nicotiana* (5) and *Lilium* (1), however, indoleacetic acid has been found to facilitate adventitious bud production from centers which were not meristematic previous to the treatment. In cabbage, although not in the other two plants, the chemical treatment merely induces the internal conditions requisite for expression of a capacity which normally rarely comes to expression. Just what determines which ones of the plants will produce shoots, however, is not known. Apparently each plant has a certain capacity for reaction, and this may be expressed in either root or shoot production or both. If shoots are produced, then the number of adventitious roots is greatly reduced.

It has been repeatedly noted by various workers that older plants or older regions of plants do not respond to applications of indoleacetic acid so rapidly nor to so great an extent as do young ones. The rate of this response in cabbage has already been noted. This is undoubtedly due to the state of maturity of the cells involved. Highly vegetative or young plants have relatively less differentiation of tissue and therefore a lesser maturity of cells than have feebly vegetative or older ones (10).

In general, the tendencies of the applications of the indoleacetic acid and the lack of nitrogen are to produce opposite effects on the cells. The former tends to cause a delay of maturity or, if the cell is already matured, first a resumption by it of that capacity for

division which is characteristic of immature cells (dedifferentiation), and then division. The lack of nitrogen, on the other hand, tends to hasten maturity of the cells, which sharply limits their capacity for division. Since most of the cells of the stems to which applications were made responded by cell enlargement or cell division or both, this resulted in a lessening of the effect of the chemical agent on the $-N$ plants. This was expressed in fewer and slower cell divisions and a hastening of maturation after cell division, and therefore in a more limited amount of tissue involvement and in a lesser growth of new organs produced, such as root primordia. The lack of nitrogen hastened the maturity so greatly that the stimulated cells often divided only once or twice before maturing. This was particularly noticeable in the cortical cells. Many of those which were stimulated to divide matured into heavy walled fibers at once, resulting in many small scattered groups of those cells in the usually parenchymatous tissue. Thus in the $-N$ plants, even though the hormone was able to overcome to a certain extent the tendency of the lack of nitrogen to hasten maturation and to prevent dedifferentiation of cells, yet the effect of the latter condition was marked.

The cells of the highly vegetative plants, most of which were in a relatively less matured state and had an ample food supply, were able to respond more readily and to a greater degree through continued divisions of their derivatives.

From a study of cellular reactions of various tissues of any one plant under different conditions, and from a comparison of the reaction of tissues of that plant with the same tissues of other plants, it is evident that, although indoleacetic acid may be the particular incitant in each case, yet it is not more than one factor in a causal complex. Reactions of the plant to it are dependent upon the hereditary constitution of the plant and the environmental factors affecting it, together with its past experience and the correlative effects of one part upon another part (11).

The fact that the unwounded plants did not react to so great an extent as the decapitated ones may be ascribed to the difficulty of penetration through the heavily cuticularized walls of the epidermis.

Summary

1. Applications of a 3 per cent mixture of indole(3)acetic acid in lanolin were made to decapitated stems and to unwounded internodes of cabbage plants grown in ordinary greenhouse soil. Similar treatment was given to plants half of which were grown under conditions of high nitrogen nutrition, the other half with a depletion of nitrogen.

2. Observations were made over a period of several weeks. The decapitated, soil-grown plants responded grossly by the production of masses of callus and root primordia.

3. All tissues of the stem responded to some extent to the treatment. The most generally responsive tissues were those of the phloem, rays, and pith. Cambium, cortex, endodermis, and xylem were moderately stimulated, while the epidermis and pericycle reacted weakly.

4. Adventitious roots were developed from ray and phloem tissue. Some internal roots were produced from pith cells adjacent to the primary xylem.

5. Near the surface of application confused mounds of callus tissue derived mainly from phloem and pith produced irregular groups of meristematic tissues in which great numbers of tracheids matured.

6. About one-third of the plants produced viable shoots, either directly from the top of the callus or laterally at about the level of adventitious root production. These lateral shoots were initiated by cell divisions either in the central or innermost cell layers of the cortex or in the rays, and always established organic union with vascular tissue of the main stem.

7. Stems treated laterally produced sunken areas through which in later stages the root primordia protruded. No mounds of callus were produced. No activity was apparent in the pith, but all other tissues reacted proportionally the same though to a less extent than in decapitated plants.

8. The reaction was slower in the $-N$ plants than in the $+N$

ones. They produced a much smaller callus, through which the root primordia rarely protruded.

9. The same tissues were involved in reactions of both +N and -N plants, although to a lesser extent in the latter. The pith and cortex of the -N plants were less active than in the +N ones, most divisions being confined to the rays, phloem, and endodermis. These, although the most active tissues in the -N stems, were still not nearly so reactive as those in the +N ones. The small amount of callus produced was less confused and showed greater vascularization than did that of the +N plants. Maturation of many cells, formerly parenchymatous, into fibers was characteristic.

10. Since the entire vascular cylinder was very unequally activated in the -N plants, far fewer root primordia were produced. These rarely grew out far enough to penetrate through cortex and epidermis.

The writer is indebted to the members of the botany department of the University of Chicago for helpful suggestions made during the course of this study.

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PITS IN THE HAPTERES OF NEREOCYSTIS

HARRIET W. ENGLERTH

(WITH FIVE FIGURES)

The presence of "sieve tubes" in the brown algae is well known; they have at least a superficial resemblance to sieve tubes of vascular plants, and occur in the center of the stipe. Surrounding them are cells which have conspicuous pits in their walls. It is with the nature of these pits that this paper is concerned.

Pitted cells were first reported by WILL (4) in *Macrocystis luxuriens*. The pits were in the lateral walls of the cells of the stipe. WILLE (5, 6), working on *Laminaria digitata*, *L. clusteni*, *L. saccharina*, and *Chorda filum*, found pits on the tangential walls of the cells and a few through the cross walls. They were closed by membranes. He called them macropores, while small holes which he thought he saw through the membranes he called micropores. ROSENTHAL (3) reported pits through the end walls of the cells of *Fucus vesiculosus*. HANSTEEN (1) found that the outer cells of *Fucus serratus* had usually only one pit between each two cells, but the outer cells of *Pelvetia* and *Sargassum* had more than one.

The plants of *Nereocystis* were gathered in Puget Sound at various times from October to April. The hapteres and the part of the stipe immediately above them were used. Small segments 1 cm. or less in length were cut and fixed in the following fluid: 100 ml. sea water, 1 gm. chromic acid, 5 ml. glacial acetic acid, and 1 drop hydrochloric acid. The pieces were subsequently dehydrated and imbedded in paraffin. Other pieces were cut freehand or with the freezing microtome. The use of alcohol and xylene apparently made the tissues hard and brittle. Dehydration and clearing with butanol by the method described by LANG (2) proved more satisfactory. Cutting fresh material either with pith or by freezing also gave good results. Both cross and longitudinal sections were made. Almost any general biological stain serves to demonstrate the pits in the walls, but it is more difficult to stain the delicate membrane which

extends across the pit. Of a large variety of stains tried, safranin, Bismark brown, and ruthenium red were most useful. A 0.25 per cent aqueous solution of safranin was used, 0.5 per cent solution of Bismark brown in 70 per cent alcohol, and 0.1 per cent slightly ammoniacal solution of ruthenium red.

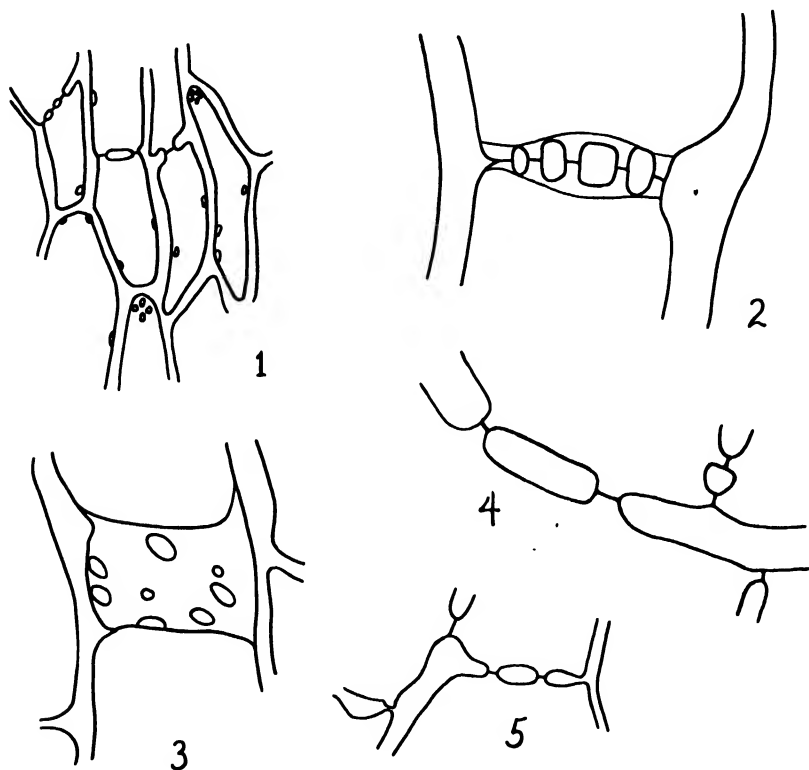
The membrane across the pit is so delicate that it easily escapes observation, especially after dehydration, which causes a marked shrinkage of the tissues. The larger pieces could be seen at times to shrink as much as 10 per cent. Soaking the cut sections in 1 per cent acetic acid counteracted this shrinking by causing a slight swelling in the walls and membranes. The sections could afterward be stained, dehydrated, and mounted in balsam without loss of detail.

Where the stalk ends and the hapteres begin, the "sieve tubes" are intermingled with the elongated cells. Some of the "sieve tubes" lie parallel with the axis of the stipe and abut on the elongated cells of the hapteres. These elongated cells (fig. 1) form the central region of each haptere. The outer cells of both the stipe and the haptere are smaller isodiametric cells.

The pits occur in practically all cells of the stipe outside of the "sieve tubes," and in all cells of the hapteres. Pits are most frequent on the tangential walls, but occur also on the radial and end walls. Each elongated cell of the stipe or center of the haptere has a circle of pits on the end wall, so that it resembles a true sieve tube except for the membranes across the pits. The smaller isodiametric cells of the stipe have fewer pits than the corresponding cells of the hapteres. A few pits were found in the inner walls of the epidermal cells.

The pits when seen in surface view appear to be holes through the walls (fig. 1), but when seen in profile (figs. 2, 4, 5) it is evident that each pit is crossed by a membrane. The cell walls of the hapteres are much thinner than those of the stipe, and the pits are smaller. Because of this any shrinkage makes it more difficult to see the pits. If the material has not been swelled by soaking in acetic acid, or if xylene was used for clearing, the walls may often be twisted, broken, or so thin that the membranes are not visible. From some of the pits the membrane (fig. 3) was torn away so that a hole resulted, but remnants of the membrane could be seen around

the edges. Many heavy stains such as anilin blue, or even safranin if destained too much, will not stain the membrane, and the pit appears as a hole, the colorless membrane being scarcely visible. Often sections are not cut exactly across the pit and the resulting appearance is that of a hole which does not extend quite through the wall.



FIGS. 1-5.—Fig. 1, elongated cells of haptere in radial section; $\times 200$. Figs. 2-5, portions of walls of elongated cells of hapteres in radial section, showing pits; fig. 5 from distal region, others from base of haptere. Drawings made with camera lucida. $\times 750$.

When examining the pit itself there often appear to be little nodules of some sort on the membrane, but on examination of many pits in both surface and profile, these nodules seem to be small particles of extraneous material gathered at each side of the pit. These, seen in surface view, may be what WILLE called micropores. The membranes of some of the pits seem to be slightly thicker in the middle.

The membrane through the pit distinguishes the pitted cells from the "sieve tubes," which are pierced by actual holes as far as is known at present.

This work was begun by the writer while at the University of Washington, and completed at Reed College. It was carried on under the direction of Dr. T. C. FRYE, University of Washington. Valuable aid and suggestions were also given by Dr. H. W. RICKETT of the University of Missouri.

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PHOTOPERIODIC PERCEPTION IN BILOXI SOY BEANS

H. A. BORTHWICK¹ AND M. W. PARKER²

(WITH ONE FIGURE)

Introduction

During recent years several investigators have concluded that flower primordia are initiated in photoperiodically sensitive plants in response to a stimulus received by the leaves and transmitted to the growing point (2, 7, 9). Many plants have been included in these various experiments, and some of the results have been summarized recently by CAJLACHJAN (3). Indirect evidence has been presented to show that a hormone "florigen" is responsible for flowering in photoperiodically sensitive plants (3). Data on many of these experiments are based entirely on the production of macroscopically visible flower buds or flowers. This practice seems open to criticism. There is some evidence (4) to indicate that flower primordia are sometimes initiated under certain environmental conditions that are not favorable to their further development into visible flowers. If this is true, it seems possible that in many cases flower primordia may be already present when photoperiodic experiments are begun. A critical morphological examination of representative plants should therefore be made before starting, and during the progress of, any photoperiodic experiments.

In the present investigation the influence of certain environmental factors on the initiation of flower primordia in the Biloxi soy bean was determined.

Observations

FLOWERING HABIT IN SOY BEAN

In previous work (1) attention has been called to the fact that the first visible flower primordia on soy bean plants appear in the axils of the fourth or fifth leaf from the apex of the main axis, and in

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corresponding positions on certain side branches. These first formed flower primordia occur on much shortened branches that arise in the axils of embryonic compound leaves.

It was found that when the treatment with short photoperiods is of only a few days' duration flower primordia are produced in a few axillary buds near the terminal, but no apparent change occurs in the terminal meristem itself. It continues to form primordia of compound leaves, in the axils of which the differentiating buds produce structures that are entirely vegetative, as was the case in the buds formed prior to the application of short photoperiods. If the treatment with short photoperiods is continued for a longer time, however, the growing point of the main axis stops forming primordia of compound leaves and forms instead primordia of simple bracteal leaves in the axil of each of which a single flower arises. This activity is the beginning of the formation of a terminal inflorescence. After a few of these bracteal leaves and flowers are produced, the growing point of the main axis ceases to elongate or to give rise to further lateral members.

After a soy bean plant has been subjected to a sufficient number of short photoperiods to cause the growing point of the main axis to begin initiating bracteal leaf primordia, it is difficult to cause the plant to resume the differentiation of purely vegetative structures at its apex by lengthening the photoperiod beyond the critical. If bracteal leaves have not yet appeared it is possible to prevent their differentiation by returning the plants to photoperiods longer than the critical.

In the following experiments induction periods have been made short so that their influence would not involve the meristem at the terminal of the main axis. Under these conditions different external environments cause differences in the number of axillary buds showing a photoperiodic response; thus a quantitative measure of the effectiveness of the treatment is available.

INTENSITY OF SUPPLEMENTED LIGHT WHICH PREVENTS INDUCTION OF FLOWER PRIMORDIA

It is recognized that artificial light of very low intensity used to prolong the photoperiod beyond the critical is effective in preventing

flowering of short day plants and in causing flowering of long day plants. The work of WITHROW (10), however, shows that the actual threshold values vary considerably in different plants. It therefore seemed desirable to obtain rather accurate data in this regard with the Biloxi soy bean.

Plants were grown on photoperiods of 16 hours, consisting of approximately 14 hours of natural light and 2 hours of Mazda, until they had three expanded compound leaves. At this time the plants were placed, for a period of 4 days, on photoperiods of 8 hours natural light plus 8 hours of Mazda light, the latter being varied in intensity from 0.6 to 100 foot candles. The controls for the experiment received only the 8 hours of natural light. At the conclusion of the 4 day treatment, all plants were returned to the original light conditions under which they were grown.

It was found when the plants were dissected 2 weeks later that initiation of flower primordia had occurred in the controls but in none of the others, indicating that 0.6 foot candle under these conditions was above the minimum necessary to prevent initiation of flower buds. Consequently, to determine the minimum another experiment was set up in which all the conditions were the same as in the first except that the intensity of supplemental light ranged from 5 to 0.01 foot candles. The results of this experiment (table 1) show that the intensities of supplementary light necessary to prevent induction lie between 1 and 0.5 foot candles. This result agrees with the first experiment in which 0.6 foot candle prevented initiation, and is also within the range found by WITHROW (10) for other plants.

It should be emphasized that these data relating to weak intensity are applicable only when low intensity light is used to extend a photoperiod of natural light beyond a critical day length.

INTENSITY OF LIGHT NECESSARY TO CAUSE INDUCTION

If induction is to be brought about by short photoperiods consisting entirely of artificial light, the intensity must be much greater than that used in lengthening photoperiods of natural light to prevent induction. Both Mazda and "sunshine" carbon arc lights were used in these experiments. Biloxi soy beans that had been growing in the greenhouse on a 16 hour photoperiod were transferred to

rooms where the temperature was held at 70° F. In one room the plants were subjected to intensities of 10, 40, and 100 foot candles of Mazda light; in the other to intensities of approximately 15, 150, and 1500 foot candles of light from a carbon arc.

The intensities of Mazda light were constant. The average intensity of light from the carbon arc was approximately constant, although there were continuous small fluctuations of very short duration. Measurements with a Weston foot candle meter showed that the intensity of light at the leaf surface of the plants subjected to

TABLE 1
EFFECT OF LOW INTENSITY MAZDA LIGHT WHEN USED TO
SUPPLEMENT AN 8 HOUR DAY OF NATURAL DAYLIGHT

LIGHT INTENSITY USED FOR 8 HOURS TO SUPPLEMENT 8 HOURS OF NATURAL LIGHT (FOOT CANDLES)	TOTAL NODES ON MAIN AXES OF 10 PLANTS	NUMBER OF PLANTS WITH FLOWER PRI- MORDIA	TOTAL BUDS ON MAIN AXES OF 10 PLANTS CONTAINING FLOWER PRI- MORDIA
5.....	187	0	None
1.....	189	0	None
0.5.....	190	2	3
0.1.....	188	8	58
0.05.....	190	10	81
0.01 to 0.02.....	190	10	88
0.0 (controls).....	188	10	102

approximately 150 foot candles did not go as low as 100 during these abrupt fluctuations. Likewise the high intensity did not go below 1000 nor the low intensity below 10. At each intensity plants were subjected to photoperiods of 8 and 16 hours, the latter serving as controls.

The plants were dissected after 5 days of treatment. Flower primordia were not initiated at any of the intensities produced by Mazda light. With the sunshine carbon arc light no flower primordia were present on the plants receiving 15 foot candles. At 150 foot candles one plant out of six had flower primordia in very early stages of development. Abundant flower buds were present on all plants that received 1500 foot candles. None of the controls in either type of light had flower primordia. These data indicate that the intensity

of light at the leaf surface must be above 100 foot candles during 8 hour photoperiods if flower primordia are to be initiated.

PHOTOPERIODIC RESPONSE WHEN TWO BRANCHED PLANTS
ARE GIVEN DIFFERENTIAL PHOTOPERIODS

In some of their early studies of photoperiodism, GARNER and ALLARD (5) found on several varieties of plants that the effects of treatment were definitely localized. For example, in short day plants the parts subjected to photoperiods longer than the critical day length remained vegetative while the parts given photoperiods shorter than the critical produced flowers. Since the production of macroscopically visible buds or open flowers was used as the basis of observation, however, it is not certain that flower primordia may not have been initiated on the parts subjected to long photoperiod. While it may seem improbable, it is at least conceivable that flower buds might be initiated under such conditions and still fail to reach macroscopic size. In our work with Biloxi soy beans it has repeatedly been found that flower buds initiated by a short induction period develop very slowly when the plants are returned to long photoperiod, and in many cases never reach macroscopic size. It has also been found in our work that soy bean plants many months old initiate flower primordia abundantly, even though they have been grown on long photoperiod continuously. The development of these into macroscopically visible buds, however, rarely occurs if the long photoperiod is maintained. As a result the plants appear vegetative, and only by careful microscopic dissection is the true condition revealed. Experiments were accordingly set up to determine whether or not initiation of flower primordia is as strictly localized as is the production of visible buds and flowers.

For this work plants having two branches of nearly equal size were used. These were obtained by decapitating young plants just below the first compound leaf. The buds in the axils of the two primary leaves then expanded. The cotyledonary buds below usually failed to start growth; if they did they were removed. These plants were kept on a 16 hour photoperiod from the time they emerged from the soil until they were used in the experiments.

With plants of this type it was possible to apply short photoperiods to one branch while maintaining the other on long photoperiod.

In this work with two branched plants, the branch placed on 8 hour photoperiod is referred to as the donor and the one on 16 hour photoperiod as the receptor, in accordance with the terminology suggested by HAMNER and BONNER (6). After the donor branches had received five short photoperiods the differential treatments were discontinued and both branches were continued on photoperiods of 16 hours. On half of the plants used the receptor branches were defoliated, just prior to the beginning of the differential light treatments, leaving only the very small leaves at the terminal. The largest of these had not begun to expand at the beginning of the experiment, and during the 5 days of photoperiodic treatment any young leaves that showed a tendency to expand were removed. After the conclusion of the 5 days no further leaves were removed from the receptors.

At the beginning of the experiment the donor and receptor branches were approximately equal in size. Dissection of similar plants on the day the donors received their first short photoperiod showed that there was an average of thirteen nodes to each branch. The individual deviations from this mean were not more than one node.

At the conclusion of the experiment every bud was dissected on both branches of all the plants. The data from these dissections (table 2) show that initiation of flower buds occurred on receptor branches only when they were defoliated. Flower primordia on these receptors were not so abundant, however, as they were on the donors. This may be accounted for by the fact that the total number of nodes differentiated on defoliated receptors was reduced by defoliation.

These results show that initiation of flower buds was localized on donors if all the leaves were present on the receptors. Initiation was not localized, however, if all the leaves were removed from the receptors. Initiation of flower buds on receptors was therefore prevented by the presence of leaves.

LOCALIZATION OF INDUCTION REACTIONS

The purpose of the following experiments was to differentiate between the action of various photoperiods when applied separately to the leaves and to the terminals of soy bean plants.

In order to give localized photoperiods to different parts of a plant, black cloth bags, made of double thickness black sateen, were employed. In direct sunlight of 10,000 foot candles they did not transmit sufficient light to activate a Weston foot candle meter which was sensitive to 0.01 foot candle. These bags were placed around a leaf or a terminal bud and fastened securely at the bottom;

TABLE 2

EFFECT OF PRESENCE OF LEAVES UPON FLOWER BUD INITIATION

NO. OF PLANTS USED	TREATMENT OF BRANCHES	TOTAL NODES IN MAIN AXES OF BRANCHES	TOTAL BUDS ON MAIN AXES OF BRANCHES CON- TAINING FLOWER PRIMORDIA
10	Donor not defoliated.....	168	37
	Receptor not defoliated.....	165	0
10	Donor not defoliated.....	172	40
	Receptor defoliated.....	158	15

the weight of the bag was supported by means of bamboo stakes. A typical setup is shown in figure 1.

The plants were grown under long photoperiod until they were just beginning to expand their third compound leaf. This leaf and the two primary leaves were removed to facilitate the procedure of applying localized photoperiods and to restrict the application of these to the two fully expanded compound leaves which still remained on the plant. The fact that the third compound leaf which was in the process of expansion was typical of neither the fully expanded leaves below it nor the unexpanded ones above constituted an additional reason for its removal. Dissection of representative plants at the beginning of treatment showed approximately fourteen nodes on the main axis, with no flower primordia present in any bud.

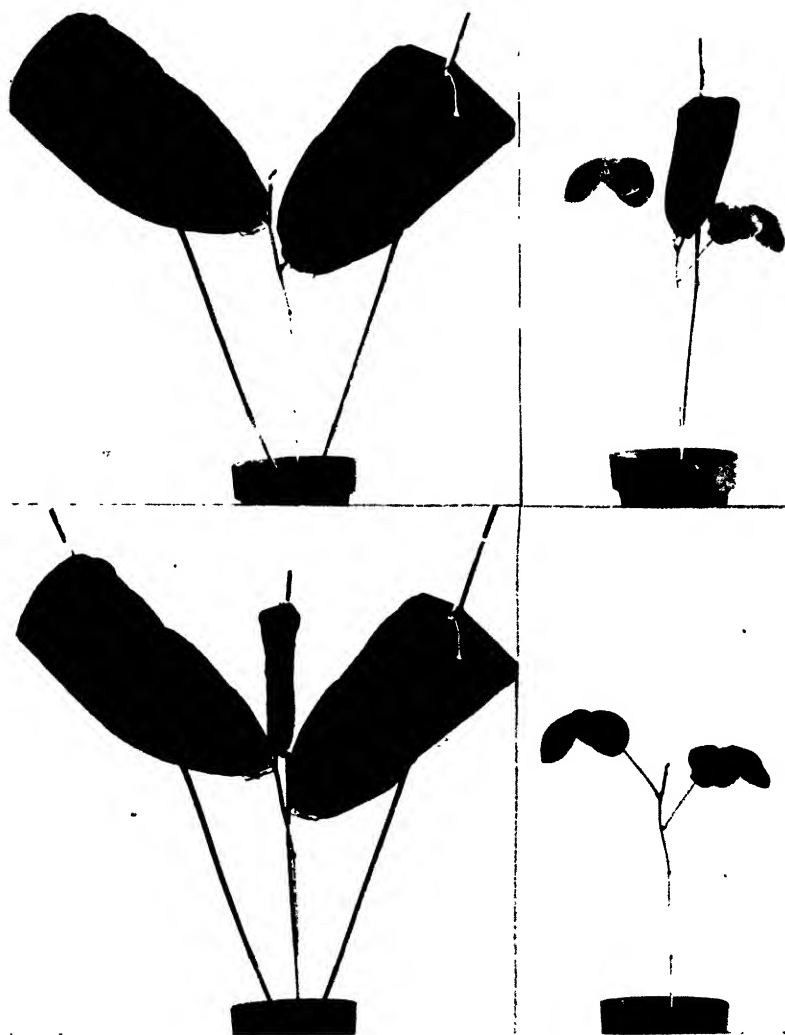


FIG. 1.—Size of plant and method of bagging used in experiments on localization of photoperiodic perception in Biloxi soy beans.

The terminal buds on three series of plants received a 16 hour photoperiod, an 8 hour photoperiod, and no photoperiod, respectively. By no photoperiod is meant that the parts concerned were kept in the dark continuously during the entire period of induction. Each series contained three lots of six plants. In one lot the leaves received a 16 hour photoperiod, in another an 8 hour photoperiod, and the remaining lot received no photoperiod. Thus photoperiods of different lengths were applied to leaves and terminal buds in various combinations. Controls were run with these lots to compare the effectiveness of black bags with large dark chambers, and also to demonstrate any effect caused directly by the removal of leaves. After three photoperiods the localized bagging was discontinued and all the plants, including the controls, were returned to a 16 hour photoperiod.

Microscopic examination was made of each node of all the plants in the experiment 18 days after their return to a long photoperiod. The controls showed that when whole plants were kept in continuous darkness for 3 days, whether in black bags or in a dark room, no flower primordia were formed. When plants were bagged from 4 P.M. to 8 A.M., however, flower buds were formed as abundantly as when the plants were kept in the darkroom during the same hours. Other controls showed that defoliation has no direct effect on initiation of flower primordia. Plants defoliated as just described and subjected to 8 hour photoperiods by means of cloth bags produced as many flower primordia as plants not defoliated but similarly bagged.

Comparison of lots in which photoperiods were applied locally to leaves and to terminals showed that initiation of flower primordia occurs only when the leaves are subjected to an 8 hour photoperiod. In all cases, when 8 hour photoperiods were applied to the leaves, induction occurred regardless of the photoperiod applied to the terminals. This reaction was not caused by the dark period alone, since no induction occurred when the leaves remained in complete darkness for the duration of the induction period and were then returned to long photoperiods.

In Biloxi soy beans the leaves are necessary to produce the stimulus causing initiation of flower primordia. This stimulus moves read-

ily in the plant, and under the conditions of this experiment causes a response in the buds near the terminal growing point, regardless of the photoperiod being applied to that region. The stimulus received during a 3 day induction period was sufficient to cause initiation of flower primordia in buds that had reached a certain stage of development. Other buds, some older and some younger, were not influenced by this short induction period and remained vegetative.

Since an 8 hour photoperiod on the leaves causes initiation, the question arises as to whether the photoperiodic response would still occur if individual leaves of the same plant received their short photoperiods at different hours of the day. Investigation of this point was made with plants that were just expanding their third compound leaf. This leaf, the two cotyledons, and the two primary leaves were removed, leaving only two compound leaves on the plant. These two leaves, which were located at the third and fourth nodes, were subjected to 8 hour photoperiods, one leaf receiving its photoperiod from 4 A.M. to noon and the other from noon until 8 P.M. Each leaf received a dark period of 16 hours following its photoperiod. In half of the plants the upper of the two leaves received its photoperiod in the morning, and in the other half in the afternoon. Controls were run in which one of the two leaves was bagged continuously and the other was subjected to 8 hour photoperiods. The upper leaf was given the 8 hour photoperiod in one lot of these and the lower in the other. In a third group of controls both leaves received 8 hour photoperiods simultaneously; in a fourth, both were subjected to 16 hour photoperiods. Treatment of the experimental plants and the controls was continued for 4 days, after which they were returned to 16 hour photoperiods.

In this experiment the combined photoperiods amount to 16 hours, which is above the critical for the Biloxi soy bean. Considered from this point of view the buds in the terminal might be expected to remain vegetative. On the other hand each leaf received an 8 hour photoperiod and a 16 hour dark period. These conditions should result in rapid initiation of flower buds, provided each leaf acts independently.

In the experimental plants one leaf of which received its 8 hour photoperiod in the morning and the other in the afternoon, flower primordia were present in the buds near the terminal regardless of which leaf received its photoperiod first. In the controls it was found that when both leaves were simultaneously subjected to 16 hour photoperiods no flowers were formed. If both were subjected simultaneously to 8 hour photoperiods, however, flower primordia were initiated. They were also initiated when either of the two leaves was kept continuously in the dark while the other was subjected to 8 hour photoperiods. These results, although preliminary, seem to indicate that flower bud formation is determined by the photoperiod given the individual leaves and not by the combined length of the photoperiods given alternately to the two leaves.

Discussion

When attempting to explain the mechanism of photoperiodism it is essential to know the morphological condition of the plants before and after differential light treatment. Induction of flower primordia may have already occurred but owing to the subsequent environment these primordia may not continue to develop. With some plants this fact would be more important than with others, since EGUCHI (4) has shown that in some cases environments suitable for induction are also suitable for development, while in others the environment associated with induction is not suitable for continued development of the primordia. In either case only plants of known morphological condition should be used for physiological studies of photoperiodism.

In the work of LOEHWING (8) with Ito San soy beans no dissection data are given, but one would infer from the fact that flowers began to appear 10 days after the start of short day treatment that their primordia must have been well established before the treatments were started. Biloxi soy beans require about 3 weeks from the beginning of induction to the opening of first flowers. Ito San soy beans grown at Beltsville on 14 hour photoperiod for 22 days initiated abundant flower primordia. Other Ito San plants grown con-

tinuously on an 18 hour photoperiod also formed flower primordia. There was no microscopic evidence of these until the plants were nearly 4 weeks old.

It is entirely possible that Ito San plants grown at a different location and at a different time of year may show a different morphological response. The results indicate, however, the extreme importance of careful morphological examination of representative plants at the beginning of all photoperiodic experiments as well as at their termination.

The results here presented show that the leaves of Biloxi soy beans are the organs of photoperiodic perception. This is in accord with the findings of other investigators with various plants. Initiation of flower buds does not occur in soy beans if they are subjected to darkness during the entire experimental period. Whether the critical reaction that leads to flower bud formation is one that occurs in light or in dark is not clear from the data thus far obtained, for it has not yet been possible with this plant to reduce the induction period to less than two short photoperiods and two long dark periods.

Induction does not occur when 8 hour photoperiods of natural daylight are extended to 16 hours by adding 8 more hours of Mazda light of intensity as low as 0.6 foot candle. This additional 8 hours of low intensity may be regarded as effective either in continuing a photochemical reaction or in preventing a reaction that requires almost complete darkness. Since it has been shown, however, that the intensity of light during the 8 hour photoperiod must be greater than 100 foot candles if initiation is to occur, it seems that in the soy bean the response is in some way influenced by conditions occurring during the light period.

The data obtained show that a short photoperiod results in a reaction in the leaves, the effects of which when transmitted to certain buds will regulate their differentiation. Several investigators have proposed that flowering in photoperiodically sensitive plants is induced by the action of a flower forming hormone. Whether the interpretation is correct or whether the responses secured are due to other causes remains to be determined.

Summary

1. When whole plants of Biloxi soy beans are subjected to 8 hour photoperiods, initiation occurs if the intensity of light during the photoperiod is above 100 foot candles. Below 100 foot candles no flower primordia are initiated.

2. When plants are given an 8 hour photoperiod of natural light supplemented by 8 hours of Mazda light, initiation occurs if the intensity of the supplemental light is below 0.5 foot candle, but does not occur if the intensity is above 0.5 foot candle.

3. The stimulus that causes initiation of flower primordia at the growing points arises in the leaves and moves to the growing plants.

4. Flower primordia may be initiated at growing points that are kept, either in complete darkness or on photoperiods above the critical, provided the leaves are kept on short photoperiods.

5. Photoperiods shorter than the critical, applied directly to growing points, have no effect on flower bud initiation. Control of initiation is exercised only through application of photoperiods of proper length to the leaves.

6. Whether the responses secured in these experiments are due to a flower forming hormone or to other causes remains to be determined.

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PHOTOPERIODISM IN RELATION TO HORMONES AS FACTORS IN FLORAL INITIATION AND DEVELOPMENT¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 496

KARL C. HAMNER AND JAMES BONNER

(WITH ELEVEN FIGURES)

I. Introduction

In 1925, GARNER and ALLARD (7) showed that, for some plants, the response to photoperiod is localized; that is, the separate portions of the plant directly subjected to the various photoperiods responded individually rather than there being a general systemic response of the entire plant. KNOTT (10) demonstrated in 1934 that the initial effect of photoperiod is received by the leaves in spinach, a long day plant. Subsequently, workers in Russia, CAJLACHJAN (2), MOSKOV (16), LUBIMENKO (13), and others, extended the work of KNOTT to other plants and confirmed his view that the leaves are the organs which receive the initial photoperiodic stimulus. Extensive studies of the movement of the effect within the plant and across graft unions have also been made. This work has recently been summarized by CAJLACHJAN (5).

Following the subjection of plants to photoperiods conducive to flowering, there seem to be produced certain effects which are capable of being transported within the plant, and across graft unions. The hypothesis has been advanced by CAJLACHJAN and others (3, 11, 12, 16) that a specific flower forming hormone is manufactured by leaves under certain photoperiods, and that this may move to various parts, resulting in floral initiation and often in subsequent development. SACHS (17) suggested that specific flower forming substances probably exist. His experiments also indicated that such a substance must be supplied from the leaves. It remains

¹ This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

to be established that the flowering response is actually controlled by a specific chemical substance.

It is desirable to bear in mind precisely what is meant by the term "flowering condition" of plants (9). This term may be divided into at least six stages: (1) induction of the flowering condition; (2) initiation of the flower primordia; (3) development of these primordia into macroscopic flowers; (4) development of the fruit; (5) gametic union; and (6) development of seeds. There may well be many hormones which influence the flowering of plants, and not only may each particular hormone be involved in a single stage of the process, but there may be many interactions as well. The present work is concerned mainly with a study of the conditions leading to the induction and initiation of flower primordia, and their possible subsequent development.

Xanthium pennsylvanicum, a cocklebur, was chosen as the principal experimental material. If grown continuously under ordinary greenhouse conditions at photoperiods in excess of 16 hours, it remains vegetative indefinitely. It is a short day plant, possesses a sharp critical period, and blooms at photoperiods shorter than 15 hours. Its floral primordia are readily recognizable under the dissecting microscope within 5 days after transfer of plants from long to short photoperiod. It possesses the advantage also that once floral primordia have been initiated under short photoperiods, they develop into flowers and fruits despite subsequent wide variations in environmental conditions. *Xanthium* grows rapidly, and if subjected to short photoperiods will flower in 14 days after emergence of the cotyledons from the soil.

II. Terminology and experimental methods

For the experiments reported here mature fruits of *Xanthium pennsylvanicum* were collected in November, and others which had passed the winter out of doors were collected in the vicinity of Chicago in early May. All were stored at room temperature in a dry place until used. The seeds were removed from the fruit, scratched slightly to rupture the seed coats, and after removal from the bur were planted within a few days in an open garden soil. Four seeds were sown in 4.5 inch pots, or 100 seeds in an ordinary greenhouse

flat. After the seedlings had attained a height of 6-10 inches, they were transplanted singly to 3.5 inch pots in order to obtain sturdy plants.

In certain experiments (section X) the seeds were planted in pure quartz sand contained in 2 gallon glazed crocks, and supplied on alternate days with 500 cc. of SHIVE's slightly modified R_2S_5 nutrient solution (18). On intervening days the plants were supplied with distilled water. Such accessory growth factors as were used were



FIG. 1.—Plants at stage of development used in most experiments. From left to right: defoliated at base, defoliated at tip, intact (not defoliated), and two branched.

added to the nutrient solution just prior to its application to the crock.

Excessively high temperatures or low humidities were minimized by ventilation of the greenhouse and frequent spraying of the walks and benches. The plants were well watered and grew vigorously throughout the experimental period. In most of the experiments (those labeled higher than X-13) the plants were 3-5 weeks old. The plants used in experiments labeled X-1 to X-13, however, were more than 6 weeks old when used, and in all experiments concerned with two branched plants, the plants were 4-6 weeks old (fig. 1). All plants were grown until used for experimentation on evenly lighted benches in the greenhouse. The natural daylight was supplemented with Mazda light of about 100 foot candles at the surface

of the leaves. These lights were burned from 7 P.M. until midnight in all experiments up to August 17, and from 6 P.M. until 2 A.M. after August 18. The photoperiod thus varied from 18.5 to 20.5 hours. Plants maintained continuously under these conditions of long photoperiod remained strictly vegetative. The term long photoperiod, as used in these experiments, means one in excess of 18.5 hours accompanied by a dark period of less than 5.5 hours. The term short photoperiod, as used in this work, refers to a photoperiod of 9 hours' duration with an accompanying dark period of 15 hours.

Great care was taken to maintain accurately the desired photoperiod. Lamps were checked daily, and plants were dissected periodically to make certain that they were in fact strictly vegetative. As a result of this checking, several thousand plants were discarded because of the accidental failure of the supplementary illumination for two nights. In every experiment also, control plants were dissected at the beginning and at the end of the experimental period. No experiment is reported in which control plants on long photoperiod or on 16 hour photoperiod were not strictly vegetative. With few exceptions, as noted in the tables, the plants subjected to a specific treatment exhibited a uniform response; that is, either all plants were strictly vegetative, or all plants possessed floral primordia or flowers at the same stage of development.

When it was desired to control accurately the photoperiod to which a group of plants was subjected, special trucks were used. During the day, in general for 9 hours, these trucks stood in an evenly lighted greenhouse. Each evening each truck was rolled into its light-tight compartment, where it remained until the following morning. In this way the plants received 9 hours of natural daylight, supplemented with artificial light of any desired duration from Mazda lamps controlled by time clocks. A series of smaller and simpler compartments were also used (fig. 2). Each evening the frames of these compartments were covered with black sateen cloth, two layers thick. These covers were removed each morning at 8 A.M. This set of seven compartments, each on a separate time clock, proved particularly useful for experiments in which it was desired to grow a few plants on each of a number of different photo-

periods; that is, for determination of critical period, for interruption of the dark period by short light periods, etc.

In the experiments in which vegetative plants were grown as close to the critical day length as possible, the 9 hours of natural daylight were supplemented by light from Mazda lamps for 7 hours, thus making a total of 16 hours, a photoperiod just in excess of the critical² (section IV). This supplementary light was given immediately after the 9 hours of daylight on certain trucks, on others it was given immediately before. These two types of 16 hour photoperiods



FIG. 2.—Type of small compartments used in experiments. The one at left has cover in place. Each controlled by separate time clock.

were used in the experiments of section IV. Plants which had been growing on a truck receiving supplementary light in the evening were removed during the day and placed on a truck receiving supplementary light in the early morning. These transferred plants then received a short photoperiod of 9 hours. The following dark period, however, was for them of 8 hours' duration. In this way the plants growing continuously on 16 hour photoperiods were given one short photoperiod without an accompanying long dark period.

² In some experiments, noted in the tables, a 15.5 hour day was used. In these experiments the controls were vegetative (15.5 hours was above the critical period).

A standard, relatively simple procedure was developed for treating one portion of a plant with short photoperiod and the rest of the plant with long photoperiod. Cardboard cans were painted inside with flat black and outside with aluminum paint. The covers of the cans were removed, slit, and provided with a central hole (fig. 3). The covers were then slipped in an inverted position on to the stem of the plant, the stem passing through the central hole. The radial slit was then sealed with adhesive tape and the cover fastened firmly with thumbtacks to a wooden frame, to which the pot was also wired (fig. 3). The body of the can could then be inverted over the plant and slipped into position in the cover. The light intensity inside such a can is less than 0.01 foot candle even in bright sunlight, and the temperature was never more than 2° F. above the temperature of the surrounding greenhouse air.

With this apparatus it was possible to obtain any combination of long and short photoperiod on different parts of the same plant. It was easy to assemble, and as many as 250 cans have been placed in position night and morning by two people, the entire process consuming about 30 minutes.

In section IV experiments are described in which plants were submitted to various night temperatures. These plants were kept in the greenhouse during their photoperiod, and then transported for the dark period to darkrooms or compartments maintained at the required temperatures. Each morning the plants were returned to the greenhouse.

In the experiment in which a temperature of 4° C. was maintained during the photoperiod, a room equipped with a cooling unit was used. This room was equipped with a carbon arc light, supplying about 900 foot candles at the leaf surface. A similar room kept at 21° C., illuminated with a carbon arc light for 10 hours per day and Mazda light for an additional 6 hours, was used for many of the experiments with cuttings as described in section X.

By a vegetative plant is meant a plant which does not possess either flowers or recognizable floral primordia, and which, if maintained continuously on long photoperiod, does not develop them. The possession of one or more flowers or floral primordia is con-

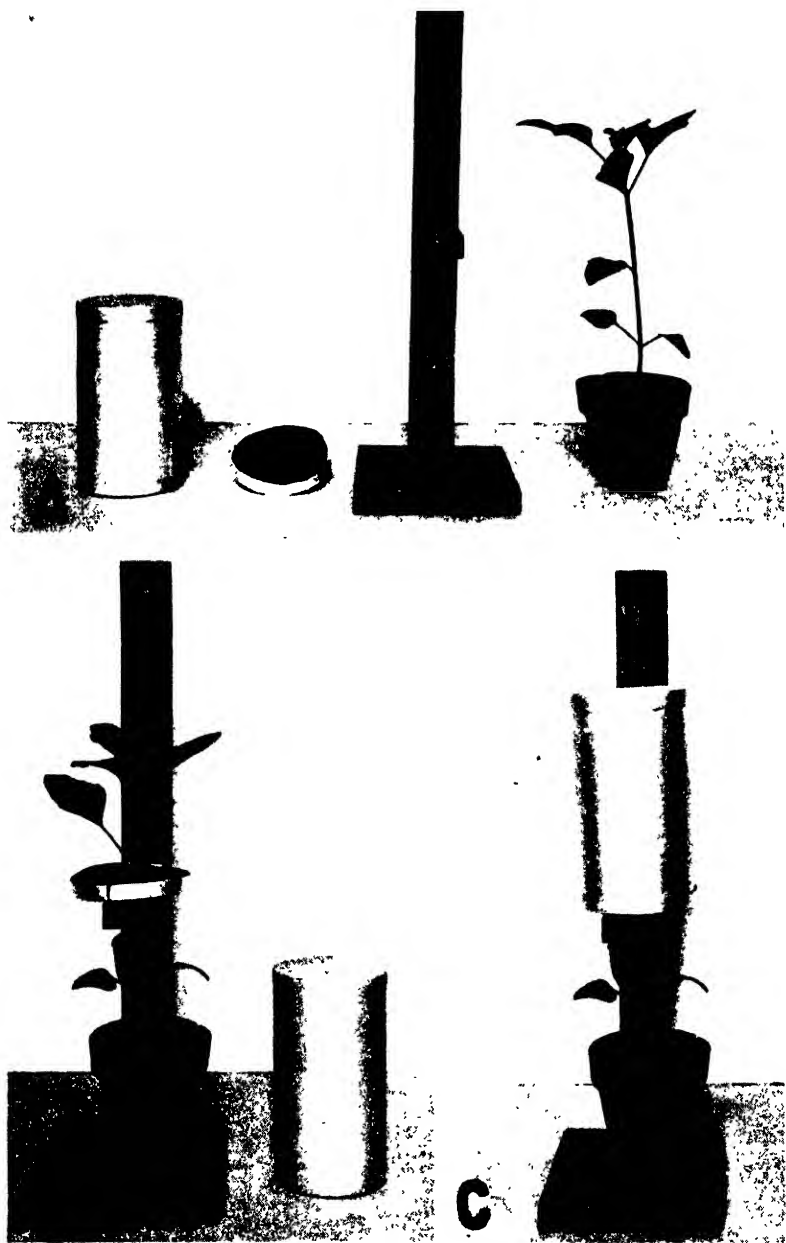


FIG. 3.—Apparatus used to subject whole or parts of plants to same or different photoperiods at same time. *A*: aluminum coated paper box and cover, wooden stand, and potted plant; *B*: plant and cover in place; *C*: box in place for darkening upper portion of plant while lower portion is still exposed to light.

sidered to represent the flowering condition. As a matter of routine, at least the terminal buds on all the plants of any experiment were dissected about 14 days after its start, in order to determine whether or not floral primordia were present. Figure 4 shows some of the stages of development recognizable upon dissection.

In cases where two large branches per plant were desired, the plants were severed at the first internode when the latter had attained a length of 0.5 to 1.0 cm., or approximately 15 days after planting the seed. As a result both cotyledonary buds expanded in many cases.

As will be discussed later, it has been found in this work that a portion of a plant exposed to short photoperiod produces an effect upon other portions of the plant not so exposed. In order to facilitate discussion, the portion of the plant on short photoperiod will be referred to as the "donor" portion, and other portions which initiate floral primordia as a result of substances received from this donor will be called "receptor" portions.

III. Evidence for a specific substance involved in floral initiation

A given plant response can definitely be linked with a given specific substance only when this substance has been isolated, chemically identified, and shown to be essential to a particular response. Experience with other plant hormones, such as auxins and vitamin B₁, has indicated that correlations within the plant are often to be attributed to chemical substances having the properties of hormones. Floral initiation might be conclusively attributed to the presence of a substance or substances if it could be shown that the effect could be linked to something which is diffusible, can pass out of one plant and back into the same or another plant, and there bring about initiation of floral primordia. Such a substance or substances might be considered as hormones. The results of the detailed experiments which follow give strong indication that hormones are involved in the initiation and development of floral primordia, flowers, and fruits by plants subjected to varying photoperiods and dark periods under certain environmental conditions.

When a *Xanthium* plant is subjected to short photoperiod, the

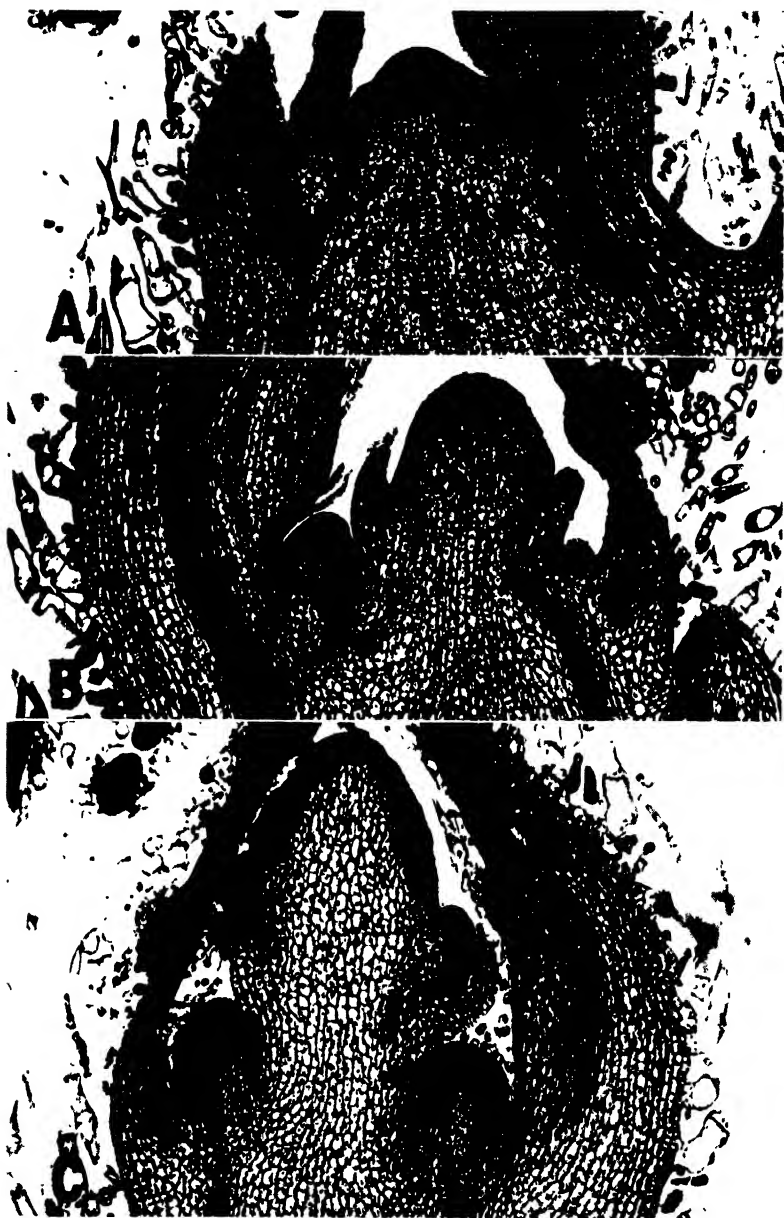


FIG. 4.—Median longitudinal sections of terminal buds of *Xanthium*. A: strictly vegetative; B: inflorescence primordium; C: later stage in development of inflorescence. Stages between B and C designated as inflorescence primordia in the tables; stages between C and macroscopic flowers designated as flower primordia.

initial stimulus to floral initiation is received by the leaves, as is shown by three types of experiments.

1. Sturdy vegetative plants were placed on short photoperiods. One lot of these was completely defoliated and the other left un-defoliated. The leafy plants were found to have large flower buds after 11 days. The defoliated plants remained strictly vegetative for 3 weeks, after which the experiment was discontinued (table 5).

2. In other experiments a single fully expanded leaf of each plant was exposed to short photoperiod while the rest of the plant received long photoperiod. These experiments, summarized in table 1, show

TABLE 1*
FLORAL INITIATION AS RESULT OF EXPOSURE OF SINGLE
LEAF TO SHORT PHOTOPERIOD

EXPERIMENT NO.	TREATMENT	DURATION OF TREATMENT (DAYS)	NO. OF PLANTS	CONDITION OF PLANT AT END OF TREATMENT
X-23 and X-41	One leaf on short photoperiod Untreated controls	18 18	32 6	mac fl & fr veg

* In this and all subsequent tables the condition of the terminal bud at the time of dissection is indicated as follows:

veg: strictly vegetative.

infl pr: inflorescence primordia.

fl pr: flower primordia.

mac fl & fr: macroscopic flowers and fruits.

Unless otherwise stated, all plants which received a given treatment were in the same stage of floral development. In all tables the term untreated controls refers to those plants which were maintained on a long photoperiod at the side of the experimental plants, whenever the latter were exposed to long photoperiod, and serve to show whether or not supplementary illumination may have failed at any time during the experiment.

that one leaf exposed to short photoperiod may be sufficient to result in the initiation of flower buds and the development of macroscopic flowers, even though the remainder of the plant is growing on long photoperiod. An example of such a plant after the leaf had received 20 short days is shown in figure 5.

3. In an experiment of another kind two branched plants were used. One branch received short and the other long photoperiod. In one lot of plants the short day branch or donor was defoliated, and in the other it was left intact. In table 2 it may be seen that if, at the time it is subjected to short photoperiods, the donor branch pos-



FIG. 5.—Development of flowers on a plant one leaf of which (shown at right of cover) has been exposed to 20 short photoperiods; remainder subjected to long photoperiods only.

sesses leaves, initiation of flower buds takes place on the donor and receptor branches. If such a short day branch had previously been defoliated, both branches would remain completely vegetative.

A number of experiments were performed in order to determine in what stage of development leaves are most effective in the perception of the photoperiodic stimulus. A group of vegetative plants was selected and divided into four lots. One lot was completely defoliated, one not defoliated in any way, one defoliated except for the young expanding leaves, and the fourth defoliated except for one fully expanded leaf. Of the last lot, the area of the expanded leaf on

TABLE 2

EFFECT OF DEFOLIATION OF BRANCH EXPOSED TO SHORT PHOTO-
PERIOD ON FLORAL INITIATION IN BRANCH EXPOSED
TO LONG PHOTOPERIOD

EXPERIMENT NO.	TREATMENT	NO. OF PLANTS	DURATION OF TREAT- MENT (DAYS)	CONDITION OF RECEPTOR BRANCH
X-37	Donor branch de- foliated	6	14	veg
	Donor branch leafy	6	14	mac fl & fr
	Untreated controls	6	14	veg

any given plant was reduced by cutting away part of it, leaving approximately 2-3 sq. cm., an area about equal to that of the young expanding leaves on other plants. All plants were subjected to short photoperiod, some plants of each lot receiving one short photoperiod (with an accompanying long dark period), other plants two, and so on. During the course of the treatment, as young leaves expanded, the plants were defoliated in order to maintain approximately the same leaf area on any given plant as was present at the start of the experiment (table 13).

Undefoliated control plants required but one short photoperiod (with an accompanying long dark period) for the subsequent initiation of floral primordia, while completely defoliated control plants remained vegetative even after ten short photoperiods. The plants which possessed a portion of one mature leaf initiated floral primor-

dia after receiving three short photoperiods. The plants having an equal area of young leaves, on the other hand, remained strictly vegetative even after seven short photoperiods. This experiment and others indicate that mature leaves are much more effective than are young leaves in the direct perception of the photoperiodic stimulus.

Since the initial photoperiodic stimulus is received by the leaves, the effects of this stimulus must be capable of transport within the plant to reach the bud or buds. A number of special experiments

TABLE 3
MOVEMENT OF STIMULUS TO FLORAL INITIATION
WITHIN THE PLANT

EXPERIMENT NO.	TREATMENT	NO. OF PLANTS	DURATION OF TREATMENT (DAYS)	CONDITION IN PART OF PLANT EXPOSED TO LONG PHOTO-PERIOD
X-2	Tip of plant on short photoperiod; base on long	12	17	fl pr
	Base of plant on short photoperiod; tip on long	12	17	fl pr
	Untreated controls	6	17	veg

concerning this movement have been performed. Localized portions of several plants were subjected to short photoperiod. On some several mature apical leaves were so treated; on others, mature basal leaves were used. The remainder of the plant in each case was kept on a 15.5 hour photoperiod. Other plants, not treated with short photoperiod, were kept on a 15.5 hour photoperiod as controls. Whether the top or the base alone was subjected to short photoperiod, flower primordia formed throughout the length of the plant. Controls remained vegetative (table 3). Thus the effect of short photoperiod moves both up and down the stem. This observation was confirmed in many other ways, perhaps the most convincing of which was the type of experiment in which two branched plants were used. One branch was placed under short

photoperiod, the other under either 16 hour or long photoperiod. Details of such experiments are given in tables 2, 11, 12, 15, and 16. In every case in which the short day and the long day branch were not defoliated in any way, it was found that the long day branch as well as the short day one initiated floral primordia and developed macroscopic flowers within 2 weeks. The stimulus for floral initiation must therefore be capable of moving down and out of the short day donor portion and up and into the long day receptor portion to the terminal bud of the latter.

That the upward movement of the stimulus probably does not depend upon a passive transport in the transpiration stream is indicated by experiments which were carried out in a nearly saturated atmosphere where transpiration would be expected to be low. Under these conditions also, leaves exposed to short photoperiod at the base of the plant resulted in the initiation of floral primordia at the tip.

The stimulus for the initiation of floral primordia is capable not only of movement within any given plant, but also from one plant to another. A considerable number of splice grafting experiments were carried out in the usual manner, a scion being splice grafted to a stock and the grafted plant placed in a chamber at high humidity and under the desired photoperiod. This method was found to be more difficult to carry out and to be of less wide applicability than the approach graft method, in which portions of the stems of two plants were shaved longitudinally to the cambium and the two shaved surfaces bound together with raffia (fig. 6). The leaves above the graft of one of the plants were then placed on short photoperiod and those of the other kept on long photoperiod. In each experiment two kinds of contacts were made. In the one the union was not interfered with in any way and the stock and scion united within 10 days. In the second, or "diffusion contact" type, a layer of lens paper was placed between the two members. Histological examination showed that the paper prevented the formation of a tissue connection for at least 14 days. In fact, in many cases the two members were found to fall apart as soon as the raffia binding was removed. Only soluble substances should be able to pass across such a diffusion contact.



FIG. 6.—Diffusion contact between two vegetative plants on June 28. Subsequent to that date, tip of plant shown above the cover was subjected to short photoperiod, the other defoliated and continued on long photoperiod. Both plants flowered and fruited. Small leaves on plant under long photoperiod developed after experiment was begun. Photograph taken July 26.

In the experiments recorded in table 4 both plants were initially vegetative. After the graft was made, one member was subjected to short photoperiod and the other to either the long or 16 hour photoperiods. The stimulus causing floral initiation apparently moved readily across an ordinary graft union, since the long day member of the graft developed floral primordia. This might be expected from the fact that the same stimulus moves so readily within any given

TABLE 4

PASSAGE OF STIMULUS FOR FLORAL INITIATION ACROSS DIFFUSION CONTACTS AND ACROSS GRAFT UNIONS. EXPERIMENTS X-6, X-12, X-13, X-17, X-34

TREATMENT	NO. OF DIFFUSION CONTACTS OR GRAFTS	DURATION OF TREATMENT	CONDITION OF RECEPTOR AT END OF TREATMENT
Approach graft.	10	12-33 days	{ 8 fl pr 2 veg
Diffusion contact.	18	12-33 days	{ 17 fl pr 1 veg
Untreated controls. . .	15 (plants)	12-33 days	veg

plant. The stimulus is also able to diffuse from one plant merely in close contact with another, indicating definitely that the stimulus is a substance or substances. This is, so far as the writers are aware, the first direct evidence which has been presented concerning this point.

IV. Floral initiation as controlled by length of dark period

It seems probable that the manufacture of the substance or substances responsible for the initiation of the flowering condition in *Xanthium* is not primarily a response to duration of the photoperiod, but rather a response to duration of the dark period.

In a preliminary experiment carried out in May, 1938, it was found that *Xanthium* plants grown continuously on photoperiods shorter than 15 hours invariably flowered, while plants grown continuously on photoperiods longer than 15.5 hours failed to initiate floral primordia. In a similar experiment carried out in August, 1938,

it was found that this critical photoperiod lay between 15.5 and 15.75 hours' daily illumination. Thus although the critical photoperiod varies somewhat with change of environmental factors, possibly in this case with changes of temperature, still it remained fairly constant during the experimental period. In order to decide whether the "critical day length" is determined primarily by the length of photoperiod or by the length of dark period, experiments were carried out using cycles of other than 24 hours' duration.

The critical photoperiod is as indicated of approximately 15.5 hours, with the critical dark period of approximately 8.5 hours. If the length of the daily illumination period is the determining factor,

TABLE 5

EFFECT OF COMPLETE DEFOLIATION UPON FLORAL INITIATION IN
XANTHIUM PLANTS EXPOSED TO SHORT PHOTOPERIOD

EXPRIMENT NO.	TREATMENT	NO. OF PLANTS	DURATION OF TREATMENT (DAYS)	CONDITION OF PLANT AT END OF TREATMENT
X-1	Completely defoliated	6	21	veg mac fl & fr
	Leafy control	6	21	

then *Xanthium* plants should initiate floral primordia on any cycle having photoperiods of less than 15 hours, and should fail to flower on any cycle having photoperiods longer than 16 hours. If length of dark period is the determining factor the reverse should be found; plants should initiate floral primordia on any cycle having dark periods longer than 8.5 hours, and should fail to flower on any cycle having dark periods shorter than 8.5 hours, irrespective of shortness of the photoperiod. Since it has been determined that *Xanthium* initiates floral primordia and flowers profusely on a 24 hour cycle, consisting of an 8 hour photoperiod and a 16 hour dark period, two other cycles were chosen in which the ratio of photoperiod to dark period was also 1:2. Thus a 12 hour cycle, consisting of a 4 hour photoperiod and an 8 hour dark period, and a 48 hour cycle consisting of a 16 hour photoperiod and a 32 hour dark period, were used. Table 6 shows that plants grown on the 12 hour



FIG. 7.—At the beginning of the experiment, the two branches of the plant were approximately equal in size, and vegetative. One (donor) was covered and received 33 subsequent short photoperiods; the other (receptor) had the older leaves at the base removed but was continued on long photoperiod. Both shoots fruited. Young leaves on receptor have not prevented initiation of flower primordia nor development of fruits (*see* fig. 8).

cycle with a short photoperiod and a short dark period remained vegetative, whereas the plants grown on a 48 hour cycle with a long photoperiod and a long dark period initiated floral primordia and flowered profusely. *Xanthium* initiates floral primordia when subjected to long dark periods even if the photoperiods are also long; it does not initiate floral primordia when subjected to short photoperiod if the dark periods are also short.

Another and more direct type of experiment supports the view that it is length of dark period which results in the initiation of floral primordia by *Xanthium*. Plants growing on the 16 hour photoperiod were given a single 40 hour dark period by substitution of a 'dark pe-

TABLE 6
EFFECT ON FLORAL INITIATION OF CYCLES OTHER THAN 24 HOURS
EXPERIMENT X-62

LENGTH OF CYCLE AND PHOTOPERIOD	NO. OF PLANTS	CONDITION OF PLANTS AFTER 15 DAYS
12 hour cycle { 4 hour photoperiod 8 hour dark period	10	veg
48 hour cycle { 16 hour photoperiod 32 hour dark period	10	mac fl & fr

riod for one 16 hour photoperiod. Similar plants were given a single short photoperiod of 9 hours, but with uninterrupted dark periods of 8 hours' duration. This was accomplished by the substitution of one 17 hour cycle for the usual 24 hour cycle (section II). On the basis of numerous repetitions of this experiment (table 7), it is evident that under certain conditions (see page 409) a single long dark period, unaccompanied by a short photoperiod, is sufficient to result in the subsequent initiation of floral primordia. One short photoperiod unaccompanied by a long dark period has been found to be completely without effect upon the initiation of floral primordia.

If a process involved in the initiation of floral primordia by *Xanthium* takes place at night, it would seem probable that it is one which is adversely affected by light. That this is the case is demonstrated by the following experiment (table 8). Vegetative plants

were removed from long photoperiod and placed on a 9 hour photoperiod of daylight in the greenhouse. This was supplemented by 6 hours of artificial light to give a 15 hour photoperiod and a 9 hour dark period, upon which *Xanthium* flowers abundantly. If, however,

TABLE 7

EFFECT OF EXPOSURE TO ONE LONG DARK PERIOD OR TO ONE SHORT PHOTOPERIOD OR TO BOTH. EXPERIMENTS X-46, X-31, X-73

TYPE OF TREATMENT	NO. OF PLANTS	FLOWERING RESPONSE
Short photoperiod plus short dark period* . .	4	veg
Short photoperiod plus long dark period† . .	18	mac fl & fr
Long photoperiod plus long dark period	10	mac fl & fr

* Other plants treated with similar results (cf. table 6).

† This treatment has always resulted in the subsequent development of flowers and fruits provided the night temperature is between 21° and 27° C. (cf. tables 9 and 10).

TABLE 8

EFFECT OF INTERRUPTION OF DARK PERIOD BY VARYING EXPOSURES TO LIGHT ON FLOWERING OF XANTHIUM. EXPERIMENTS X-43, X-60, X-71; UNTREATED CONTROLS ALL VEGETATIVE

LENGTH OF PHOTO-PERIOD (HOURS)	LENGTH OF DARK PERIOD (HOURS)	LIGHT EXPOSURE DURING DARK PERIOD	NO. OF PLANTS	CONDITION OF PLANTS AT END OF TREATMENT
15	9	None	12	mac fl & fr
15	4.5+4.5	1 minute after 4.5 hours	10	veg
9	15	None	10	mac fl & fr
9	6.5+8	30 minutes after 6.5 hours	10	{ 8 fl pr 2 veg
9	5+5+5	30 minutes every 5 hours	10	{ 8 veg 2 infl pr
9	5+5+5	5 minutes every 5 hours	17	fl pr

the 9 hour dark period was divided into two 4.5 hour periods by exposure to artificial light (of 150 foot candles at the leaf surface) of one minute duration, the plants remained strictly vegetative.

In other experiments a 9 hour photoperiod was given and the 15 hour dark period was interrupted at 5 hour intervals, as shown in

table 8, by varying lengths of exposure to artificial light of 150 foot candles at the leaf surface. Control plants on 9 hour photoperiod and with an uninterrupted dark period of 15 hours all developed macroscopic flowers within 13 days. Of the plants which received one-half hour of artificial light at 5 hour intervals during the dark period, only two plants out of ten initiated flower buds. When only 5 minutes' exposure to light was given every 5 hours during the 15 hour dark period, all the plants initiated floral primordia. If the 15 hour dark period was interrupted by only one half-hour exposure to light (dividing the dark period into one 8 hour and one 6.5 hour periods), eight out of ten plants initiated floral primordia. Plants which received a 9 hour dark period remained strictly vegetative when a 1 minute period of exposure to light was given in the middle of the dark period. Thus at least thirty times as much light is required to negate the effect of a 15 hour dark period as is required to negate the effect of a 9 hour dark period.

Still another line of evidence supports the view that certain critical reactions involved in the initiation of floral primordia in *Xanthium* take place during the dark period. The temperature during the dark period greatly influences its effectiveness in relation to the initiation of flower buds. To test this, plants were removed from long photoperiod and given an 8 hour photoperiod at greenhouse temperature, which during the course of the experiments varied from 20° to 30° C. In the evening these plants were placed in the dark at temperatures ranging from 4° to 38° C. The following morning the plants were returned to the greenhouse, and a few of them from each lot returned to long photoperiod. This was repeated for ten successive dark periods. The results of one of three experiments are shown in table 9. All experiments gave essentially the same results. Plants given one short photoperiod at average greenhouse temperature, and given one long dark period at temperatures between 21° and 32° C., developed floral primordia after being returned to long photoperiod. Thus during one long night sufficient floral initiation substance may accumulate to result in the initiation of floral primordia. If the temperature during the dark period is maintained at 4° C., seven long dark periods are required to achieve the same result. Temperature

ranges between 4° and 21° C. could not be maintained accurately, but the experiment showed that the higher the temperature up to 21° C. the fewer the dark periods required. The temperature coefficient of the dark process cannot be determined precisely from this experiment, but in any case it is high, with a Q_{10} of approximately 3 and thus of an order which might well be expected of a biochemical process. Table 9 also shows that plants subjected to a temperature of 38° C. during the dark periods fail to initiate floral primordia even after four long dark periods. The optimum temperature for the most

TABLE 9

EFFECT OF VARYING TEMPERATURE DURING DARK PERIOD UPON FLORAL INITIATION BY XANTHIUM. EXPERIMENTS X-86 AND X-55. PLANTS AT GREENHOUSE TEMPERATURE DURING INTERVENING 9 HOUR PHOTOPERIODS. UNTREATED CONTROL PLANTS VEGETATIVE

TEMPERATURE (°C.)	NUMBER OF DARK PERIODS									
	1	2	3	4	5	6	7	8	9	10
4.....	veg	veg	veg	veg	veg	veg	veg	infl pr	infl pr	infl pr
21.....	infl pr	infl pr	infl pr	infl pr	infl pr
27.....	infl pr	infl pr	infl pr	infl pr	infl pr
32.....	9 veg 2 infl pr	infl pr	infl pr	infl pr	infl pr
38.....	veg	veg	veg	veg

rapid response to long dark period lies therefore between 21° and 32° C.

In contrast to the striking effect of varying temperature during the dark periods, varying temperature during the photoperiods exerts but little effect on the initiation of floral primordia, as is shown by the following experiment. Plants were subjected to a cycle of 8 hour photoperiods and 16 hour dark periods. The temperature during the dark periods was maintained at approximately 24° C. Some of the plants were exposed to a temperature of 4° C. during the photoperiod by placing them for 8 hours each day in a temperature-controlled room containing a carbon arc lamp which supplied an intensity of illumination of approximately 900 foot candles at the leaf surface. The remainder of the plants were subjected

to photoperiods at greenhouse temperatures of 20° to 30° C. Table 10 shows that floral primordia were initiated after exposure to one long dark period whether the preceding photoperiod was maintained at 4° or at 20°–30° C. Although low temperature during the photoperiod was thus without significant effect on the initiation of floral primordia, there was a marked decrease in the rate of subsequent development of the primordia.

There can be but little question that the flowering response of *Xanthium* to short photoperiod depends primarily upon reactions directly related to the dark period. It may be that the reactions

TABLE 10

EFFECT OF LOW TEMPERATURE DURING PHOTOPERIOD ON FLORAL INITIATION BY XANTHIUM. PLANTS AT 24°C. DURING INTERVENING 16 HOURS DARK PERIOD (X-80). UNTREATED CONTROL PLANTS VEGETATIVE. DISSECTION AFTER 16 DAYS

TEMPERATURE OF PHOTOPERIOD	NO. OF PLANTS IN EACH TREATMENT	NO. OF SHORT PHOTOPERIODS BEFORE PLANTS WERE AGAIN SUBJECTED TO LONG PHOTOPERIOD									
		1	2	3	4	5	6	7	8	9	10
4° C. Approximately 24° C. (greenhouse)	5	1 veg 4 infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr	infl pr
	5	infl pr	fl pr	fl pr	fl pr	fl pr	fl pr	fl pr	mac fl & fr	mac fl & fr	mac fl & fr

leading to the manufacture of a floral initiating substance are adversely affected by low temperature. At least the responses of *Xanthium* indicate that, in any critical study of the relationship between temperature and photoperiodism, it is necessary to know the ranges of temperature during the dark period and during the photoperiod.

V. Nature of inhibition and localization

It has been shown in section III that if a portion of the *Xanthium* plant is subjected to short photoperiod, floral primordia are also initiated on other portions of the same plant although such portions

may be subjected during this same time to long photoperiod. This result might seem to disagree with the reports on localization of the flowering response in other plants (7). Undeveloped *Xanthium* plants, in fact, do not ordinarily exhibit the strict localization of response to short photoperiod which is found with undeveloped chrysanthemum plants. During the course of the present work a considerable number of experiments were carried out with chrysanthemum. The results were in harmony with those of others (2), namely, that if a portion of a leafy plant was subjected to short photoperiod, macroscopic flowers developed only on such portion,

TABLE 11

EFFECT OF VARIOUS TYPES OF DEFOLIATION ON INITIATION OF FLORAL PRIMORDIA BY RECEPTOR BRANCH. EXPERIMENTS X-24, X-37, X-82.
25 UNTREATED CONTROLS ALL VEGETATIVE

TYPE OF DEFOLIATION OF RECEPTOR BRANCH	UNDEFOLIATED	DEFOLIATED		
		COMPLETELY	EXCEPT FOR TWO MATURE LEAVES	EXCEPT FOR YOUNG EXPAND- ING LEAVES
Condition of receptor after 14 days.....	infl pr 25	infl pr 24	veg 24	fl pr 26
Total plants.....				

while flowers completely failed to develop on the leafy portion subjected to long photoperiod. An attempt was made therefore to determine whether a localized response to short photoperiod might be obtained with *Xanthium*, and if so, under what conditions.

In these experiments, summarized in table 11, two branched plants were used. The donor branches of all of them were not defoliated nor treated in any way except that they were maintained on short photoperiod. All the receptor branches were maintained on long photoperiod, but on different plants they were subjected to four types of treatment: (1) complete defoliation; (2) removal of the young expanding leaves at the tip, with continued removal of these as additional leaf primordia expanded; (3) removal of only the fully expanded leaves; or (4) not defoliated. Table 11 shows that only under one condition was a localized response to

short day obtained, namely, when the young expanding leaves had been removed from the receptor and only the fully expanded ones remained on it (fig. 8). Under these conditions floral primordia were initiated only on the donor whereas the receptor remained strictly vegetative. In all other cases, whether the receptor was completely defoliated or left leafy, floral primordia were initiated on both donor and receptor. If young leaves only were present on the receptor, floral primordia were initiated on it, and these developed into mature flowers and fruits more rapidly than did those on receptors which were either leafy or completely defoliated.

These effects resulting from the presence of immature and mature leaves on receptor branches which receive their stimulus for floral initiation from donor branches subjected to short photoperiod is in direct contrast to the condition prevailing when immature or mature leaves receive the photoperiodic stimulus by being directly subjected to a short photoperiod. In the latter case flower primordia are initiated only if mature leaves are exposed, and are not initiated if immature leaves only are present. The cause of this difference in behavior is not known. At present, largely as a matter of convenience, the failure to initiate and develop floral primordia on receptor branches where only mature leaves are present is referred to as an inhibitory effect of such leaves. The tendency to initiate floral primordia and the rapid development of these into flowers and fruits on receptor branches in the presence of young leaves is referred to as a promotive effect of such leaves.

This inhibitory effect of fully expanded leaves, and the promotive effect of young expanding leaves, on floral initiation on receptor branches were demonstrated repeatedly in separate experiments, as indicated in tables 11 and 12. The inhibitory effect of fully expanded leaves does not appear to depend upon the extent to which the length of the photoperiod exceeds the critical, but does depend upon the area of such leaves present upon any given branch or plant. This was shown in a series of experiments as follows: Donor branches of 70 two branched plants were subjected to a 9 hour photoperiod. The receptor branches of 35 of these were subjected to a 16 hour photoperiod while the receptors of the remaining 35 were subjected to a 22



FIG. 8.—At the beginning of the experiment this plant was similar to the plant in fig. 7. The donor was treated in exactly the same manner, but the receptor had the young instead of the mature leaves removed, and was then continued on long photoperiod. The old leaves have prevented initiation of flower primordia and development of fruits on receptor but have exercised no measurable influence in development of flowers and fruits on donor.

hour photoperiod. Thus the first set of receptors received a photoperiod only one-half hour longer than the critical, while the latter set received a photoperiod 6.5 hours longer than the critical, and the dark period in the one was four times as long as in the other. Table 12 shows that if one-half or more of a fully expanded leaf is allowed to remain on the otherwise defoliated receptor, the receptor branch remains strictly vegetative both under a 16 hour and under a 22 hour photoperiod. One-fourth or less of a fully expanded leaf exerts no inhibitory effect upon the initiation of floral primordia under either condition.

TABLE 12

EFFECT OF VARYING LEAF AREA AND VARYING PHOTOPERIOD ON INHIBITION OF FLORAL INITIATION BY MATURE LEAVES. DONOR BRANCHES UNDEFOLIATED SUBJECTED TO 9 HOUR PHOTOPERIODS. PLANTS DISSECTED AFTER 14 DAYS; 10 UNTREATED CONTROL PLANTS ALL VEGETATIVE

PHOTOPERIOD TO WHICH RECEPTOR WAS EXPOSED (HOURS)	UNDEFOLIATED	NATURE OF LEAF ALLOWED TO REMAIN ON OTHERWISE DEFOLIATED RECEPTOR					COMPLETELY DEFOLIATED
		2 MATURE LEAVES	1 MATURE LEAF	$\frac{1}{2}$ MATURE LEAF	$\frac{1}{4}$ MATURE LEAF	1 EXPANDING LEAF $\frac{1}{4}$ " LONG	
16	infl pr	veg	veg	veg	infl pr	fl pr	infl pr
22	infl pr	veg	veg	veg	infl pr	fl pr	infl pr

Since in the case of receptor branches the inhibitory effect of fully expanded leaves upon floral initiation is in opposition to the promotive effects of young expanding leaves, an experiment was performed in which only one leaf of an intermediate age was allowed to remain on the receptor. This leaf was initially approximately three-quarters of an inch in length and by the end of the 2 weeks' experimental period had developed into a fully expanded leaf. All younger leaves were removed as they began to expand. The single developing leaf, as may be seen in table 12, exerted the promotive effect characteristic of young leaves, and this effect does not depend exclusively upon the youngest leaves.

It has been shown that, under certain specific conditions, leaves on long photoperiod are capable of suppressing the initiation of floral

buds. The question arises as to whether or not this inhibitory effect is localized. In the experiments given in tables 2, 11, 12, 15, and 16, it was found that on two branched plants the number of floral primordia initiated and the rate of their subsequent development on the donor branch were entirely unaffected by any treatment given

TABLE 13

EFFECT OF APPROXIMATELY EQUAL AREAS OF YOUNG EXPANDING LEAVES OR OF FULLY EXPANDED LEAVES ON INITIATION OF FLORAL PRIMORDIA BY XANTHIUM PLANTS SUBJECTED TO SHORT PHOTOPERIOD (X-77). UNTREATED CONTROLS VEGETATIVE. DISSECTION AFTER 17 DAYS

PLANTS DEFOLIATED EXCEPT FOR	NO. OF SHORT PHOTOPERIODS						
	1	2	3	4	5	6	7
Portion of one mature leaf of about 2 sq. cm. area...	veg	veg	fl pr	fl pr	fl pr	fl pr	fl pr
One young expanding leaf about 2 sq. cm. area...	veg	veg	veg	veg mac fl	veg	veg	veg
Undefoliated control.....	infl pr	fl pr	fl pr	mac fl & fr

the receptor branch. In these experiments the effect of the leaves on long photoperiod has been apparently confined to the branch exposed to long photoperiod.

VI. Relative response of various buds on a plant

On any given plant placed upon short photoperiod, a greater total number of flowers and fruits will develop rapidly if the terminal bud is removed. When the terminal bud of a vegetative plant is removed, many lateral buds start to expand at once, and these buds immediately develop flowers and fruits if the plant is on short photoperiod. A similar result is obtained if, instead of removing the terminal bud, the young expanding leaves are removed.

The several buds on any given donor or receptor do not respond alike. In general the buds on the donor branch respond more rapidly and more of them develop into mature fruits than do those of the receptor branch. On any plant, however, the terminal growing point of any branch responds more rapidly than the primordia of axillary buds. Thus if the terminal bud of the donor branch is removed at

the start of treatment, the terminal growing point of the receptor branch responds more rapidly than do the axillary buds of the donor.

Since the inhibition of the expansion of axillary buds seems to be associated with the presence of young expanding leaves near the tip of the axis, as described, and since the presence of young expanding leaves on the receptor of a two branched plant seems to produce some promotive effect upon the development of flowers and fruits by the receptor branch (section V), it is possible that young leaves, through their ability to supply auxin, produce both results. To test this possibility the following experiments were carried out: An attempt was made to replace by the use of indoleacetic acid the promotive effect of young expanding leaves on floral initiation. The receptor branches of two branched plants were defoliated either completely, or by removal of the young expanding leaves at the tip. Indoleacetic acid in lanolin paste (1 and 0.2 per cent) was then applied to the petiole stumps of the youngest three leaves. Indoleacetic acid applied in these high concentrations was completely without effect on either the initiation of floral primordia or on the development of flowers. Receptor branches, with mature leaves only, remained vegetative even if supplied with indoleacetic acid, as described, and flowers did not develop more rapidly on completely defoliated branches so treated than on untreated defoliated branches.

In another experiment similar high concentrations of indoleacetic acid as a lanolin paste were applied along the entire length of the stem, and the number of long dark periods necessary for the initiation of floral primordia was compared with the number necessary for untreated control plants. It was found that the untreated control plants, those treated with plain lanolin paste, and those treated with 0.2 per cent indoleacetic acid, all flowered on subsequent long photoperiod if they were subjected to one or more long dark periods. Plants treated with 1 per cent indoleacetic acid in lanolin, however, flowered only if given two or more long dark periods and not when given one dark period. No effect upon the relative sensitivity of the various buds was found. Thus, although auxin may play some role in the inhibition of flower bud initiation, still this effect is not

marked, when indoleacetic acid is applied. In numerous experiments carried out as described in section IX, indoleacetic acid was also without activity in initiating floral primordia in *Xanthium*. What relation this may bear to the well known effect of indoleacetic acid in suppression of bud development was not investigated.

VII. Nature of induction

In the preceding sections consideration has been given mainly to the initiation of floral primordia by *Xanthium*. It has been observed during these experiments, as well as in those of others (4), that initiation of floral primordia by the influence of suitable photoperiod is accompanied by other striking aftereffects when the plant is subsequently transferred to other photoperiods.

If a vegetative *Xanthium* plant is removed from long photoperiod, subjected to one long dark period under suitable environmental conditions (section IV), and returned to long photoperiod, this plant will initiate floral primordia and will develop macroscopic flowers after approximately 14 days. During the single long dark period no histologically detectable change occurs in the plant. Nevertheless some change has been brought about, a change which results in its flowering when grown subsequently on long photoperiod. This phenomenon, which is essentially one of photoperiodic aftereffect, may be referred to as photoperiodic induction. Induction in this sense is not the initiation of floral primordia per se, but is rather the change within the plant which takes place in relation to an alternation of photoperiods and dark periods which results in the initiation of floral primordia. Under suitable conditions of temperature this change may be brought about in *Xanthium* by subjection to one or more long dark periods. Subsequent flowering results whether the photoperiods following the long dark period are long or short. Induction appears to be associated with some alteration in metabolism, and experiments have been performed to investigate the nature of this alteration.

Plants which have undergone induction continue to supply the floral initiation substance when subsequently subjected to long photoperiods. This was demonstrated by the use of plants which

had been induced by exposure to 24 short photoperiods, and which were approach grafted to defoliated vegetative plants. Both plants were then allowed to remain on long photoperiod. The results are shown in table 14.

This ability of induced plants to continue to supply the floral initiation substance when such plants are returned to long photoperiod, might be attributable to (a) the storage of reserves of it during the exposure to short photoperiod and usage of this supply during the following long photoperiods, or (b) an alteration of the metabolism of the plant such that it manufactures the substance even

TABLE 14

PLANTS INDUCED BY EXPOSURE TO SHORT PHOTOPERIOD CONTINUE TO SUPPLY FLORAL INITIATION SUBSTANCE EVEN WHEN SUBSEQUENTLY SUBJECTED TO LONG PHOTOPERIOD. UNTREATED CONTROLS VEGETATIVE

EXPERIMENT NO.	TREATMENT	NO. OF GRAFTS	TIME OF OBSERVATIONS	CONDITION OF RECEPTOR
X-12 and X-17	Photoperiodically induced donor grafted to vegetative receptor	8	14-22 days	{ 6 infl pr 2 veg

when subjected to long photoperiods. A final decision between these two alternatives is not possible at the present time, but a number of considerations indicate that the latter may be the more probable, as is illustrated by the following experiment: Small vegetative *Xanthium* plants were subjected to seven short photoperiods during and after which periods they initiated floral primordia and bloomed. They were then grown under continuous illumination for 7 months. After this they continued to receive long photoperiods for a total duration of more than one year. During this period the plants increased in size 100 or more times, and produced many new shoots. They also continued to produce flowers, although new axillary shoots occasionally developed vegetatively for a time. It would seem unlikely that floral initiation and flower development substances stored in the young plants during the seven consecutive short photoperiods could be responsible for this striking aftereffect. It seems more prob-

able that these plants continued to manufacture the substances in question during the exposure to long photoperiods. These plants have in effect become indeterminate or everblooming with respect to photoperiod.

It has been a common experience during the course of the present work to find that the development of floral primordia into mature flowers is much slower for a plant which has been induced by one long dark period, than for one given a succession of long dark periods. Thus the rapidity with which a plant supplies the sub-

TABLE 15

EFFECT OF VARYING EXPOSURES OF DONOR BRANCH TO SHORT PHOTOPERIOD ON DEVELOPMENT OF FLOWERS AND FRUITS ON RECEPTOR AFTER REMOVAL OF DONOR. DISSECTION AFTER 21 DAYS

EXPERIMENT NO.	NO. OF SHORT PHOTOPERIODS TO WHICH DONOR WAS EXPOSED	NO. OF PLANTS	SUBSEQUENT DE- VELOPMENT OF FLOWERS AND FRUITS ON RECEPTOR
X-53.....	2	5	veg
X-53.....	4	5	fl pr
X-53.....	8	5	fl pr
X-25.....	11	4	fl pr
X-24.....	27	20	mac fl & fr

stances necessary for floral development seems to depend to a certain extent upon the length of the induction period.

The ability to supply these substances necessary for floral development seems to be transferable from one branch to another. This has been demonstrated in several experiments in which a number of lots of two branched plants were used. In these the donor branches of each lot were subjected to a definite number of short photoperiods. Then the donor branch was severed from the plant, while the receptor branch was in every case maintained under conditions of long photoperiod. The development of flowers and fruits on these receptor branches was then observed at intervals. The results of three such experiments are given in table 15. If the donor received but two short photoperiods before it was removed, the receptor remained strictly vegetative, even after 30 days. If, however, the



FIG. 9.—Receptor branch of two branched plant of which the donor received 27 short photoperiods and was then removed. At the time of removal, all macroscopic flowers and fruits were removed from the receptor (fruits shown have developed subsequent to that time). Photograph taken 40 days after removal of donor.

donor received four short photoperiods and was then removed, floral primordia were subsequently initiated on the receptor, and these primordia continued to develop somewhat further, although slowly. In one experiment the donor received twenty-seven short photoperiods before it was removed. During this period the receptor had developed macroscopic flower and fruits. All such flowers and fruits were removed from the receptor when the donor was cut off. Forty days later numerous flowers and fruits had again developed on these receptor branches, which had themselves never been subjected to short photoperiod. An example of such a receptor is given in figure 9. It is evident that if the donor branch is exposed to a sufficient number of short photoperiods the receptor may behave in some respects as if directly photoperiodically induced; that is, changed in a manner which results in the continued production of flowers and fruits even when it is subjected to uninterrupted long photoperiods.

Such indirect induction at least partially depends upon the leaves of the donor and is accelerated if the buds on the donor are removed (fig. 10*B*). The plants shown are of a series whose donors received eleven short photoperiods. At the end of this time all the receptor branches possessed floral primordia. The plants were then divided into four lots and the donor branches of any given lot were subjected to one of the following treatments: (1) severed from the receptor, (2) disbudded and exposed to long photoperiod, (3) defoliated and exposed to long photoperiod, and (4) left intact and exposed to long photoperiod. In figures 10 and 11 a typical receptor of each group is shown after a development of 40 days following the respective treatments. In the cases where the buds of the donor were removed but the leaves allowed to remain, the receptor possessed numerous large fruits. Where the donor had been removed, the buds of the receptor had developed but little. The other two cases exhibit intermediate development of flowers on the receptor. Removal of the buds on the donor at the end of the short photoperiod treatment thus greatly accelerates the subsequent development of flowers and fruits on the receptor.

In another experiment 40 two branched plants were used. These



FIG. 10. —*A*: receptor branch of two branched plant whose donor received 11 short photoperiods and was then immediately removed. *B*: receptor branch of a two branched plant whose donor branch after having received 11 short photoperiods, was not removed but had its terminal and all its axillary buds removed. Note that although both receptors were grown continuously under long photoperiod, *A* has macroscopic flowers only and *B* well developed burs. Photograph taken 41 days subsequent to beginning of treatment.

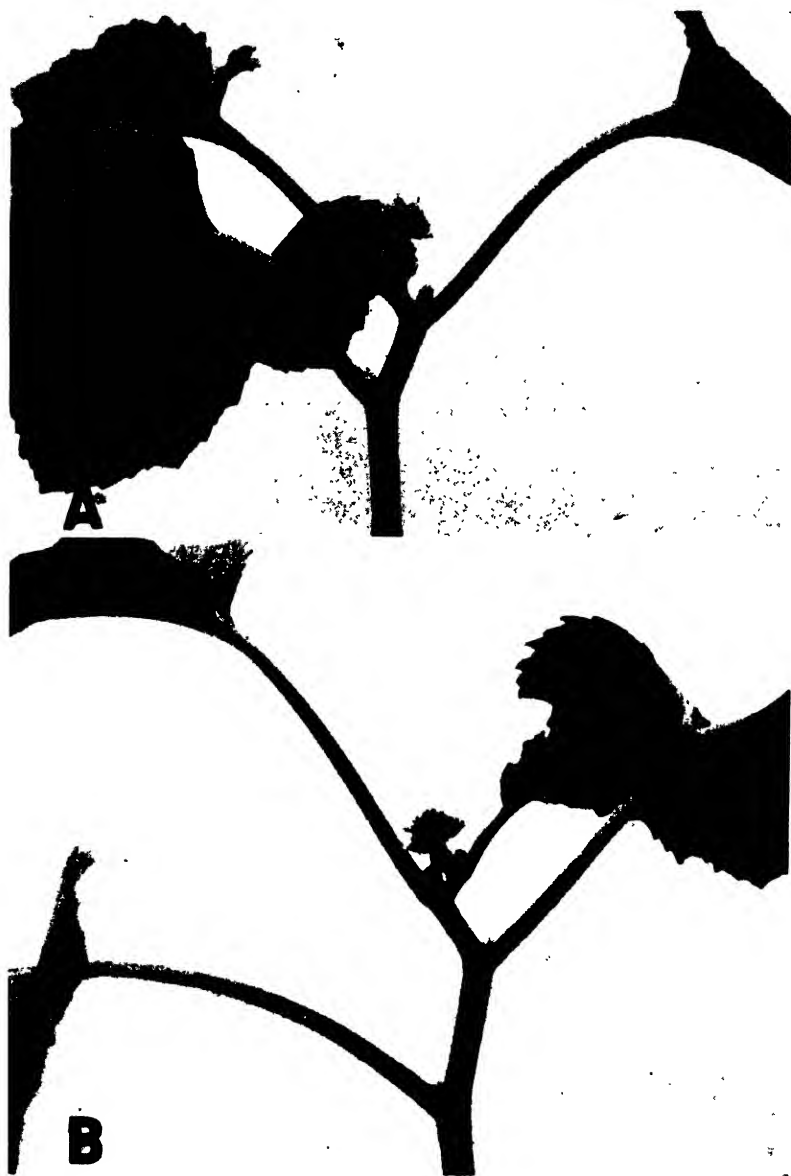


FIG. 11.—*A*: receptor branch of two branched plant whose donor received 11 short photoperiods and was then defoliated. *B*: receptor branch of two branched plant whose donor branch received 11 short photoperiods and was not subjected to further treatment other than being continued on long photoperiod. Note that *A* and *B* are intermediate with respect to floral development between *A* and *B* of fig. 10.

were divided into two groups; the donor branches of one group received two short photoperiods and those of the second group received four. After the treatment of a given group with short photoperiod, each group was divided into four lots of five plants each, and the donor branches of any given lot were subjected to one of the following treatments: (1) severed from the receptor, (2) disbudded and exposed to long photoperiod, (3) defoliated and exposed to long photoperiod, or (4) left intact and exposed to long photoperiod. The results are recorded in table 16. Regardless of the subsequent treat-

TABLE 16
EFFECT OF VARIOUS TREATMENTS OF DONOR ON DEVELOPMENT OF
FLOWERS AND FRUITS BY RECEPTOR. EXPERIMENT X-53

TREATMENT OF DONOR		NO. OF PLANTS	DEVELOPMENT OF FLOWERS AND FRUITS ON RE- CEPTOR AFTER 21 DAYS
Two short photoperiods then placed on long photoperiod and	Cut off	5	veg
	Defoliated	5	veg
	Disbudded	5	fl pr
	Left intact	5	fl pr
Four short photoperiods then placed on long photoperiod and	Cut off	5	fl pr
	Defoliated	5	fl pr
	Disbudded	5	mac fl & fr
	Left intact	5	fl pr

ment of the donor, all the receptors of the plants on which the donor received four short days initiated floral buds. All receptors remained strictly vegetative on those plants whose donors were removed or defoliated and placed on long photoperiod after these donors had received only two short photoperiods. The receptors initiated floral buds in those cases where the donors received two short photoperiods and were subsequently exposed to long photoperiods, either intact or disbudded. The difference in floral development in these cases indicates that leaves of the donor which have been exposed to an induction period of short photoperiod continue to supply substances which bring about initiation and development of floral primordia in the receptor, even after these leaves are subsequently placed on long photoperiod.

VIII. Attempts to determine nature of floral initiation substance

On the basis of the observation that the floral initiation substance *diffuses* from a plant subjected to short photoperiod into a vegetative plant exposed to long photoperiod and brings about the initiation of floral primordia by the latter, attempts were made to extract the floral initiation substance from leaves which had been subjected to short photoperiod, and by the use of such extract to bring about floral initiation in vegetative plants or cuttings.

Several test methods were employed for supplying such extracts to vegetative plants maintained on long photoperiods. A desirable

TABLE 17
RESPONSE OF XANTHIUM CUTTINGS TO LONG AND
SHORT PHOTOPERIODS

EXPERIMENT NO.	TREATMENT	NO. OF CUTTINGS	CONDITION OF CUTTINGS AFTER 14 DAYS
X-14 and X-29	Short photoperiod Long photoperiod 16 hour photoperiod	15 10 10*	fl pr veg veg

* Approximately 4500 cuttings have been subjected to 16 hour photoperiod and have remained strictly vegetative in other experiments.

method would be one in which vegetative shoots kept on days longer than the critical would be able to take up the solution to be tested. It is also necessary that the control plants remain vegetative. Cuttings were used most extensively as test material. Sturdy vegetative plants 3-4 weeks old were severed above the cotyledonary node, and defoliated below the uppermost partly expanded leaf. These cuttings were tied in groups of four or five and placed in 20 cc. shell vials containing the solutions to be tested. It is shown in table 17 that if such cuttings are placed in water and exposed to short photoperiod, they respond readily in the production of floral primordia and of macroscopic flowers. When similar cuttings were subjected to 16 hour photoperiod or to long photoperiod they remained strictly vegetative. Cuttings respond to short day with floral initiation just as do intact plants, and it would seem justifiable to make use of such

cuttings as test material for the detection of the floral initiation substance.

In all of the cutting tests to be described, the cuttings were maintained under either a 15.5 or a 16 hour photoperiod. The humidity was adjusted so that approximately 10 cc. of solution per four cuttings per day was lost by transpiration. That solutions or extracts supplied to the cuttings were actually taken up was shown by the marked toxic effects of certain solutions upon the upper leaves of the cuttings, and by the marked effects which certain solutions exerted on vegetative growth (stem elongation, rooting, etc.). In most cases the solutions were renewed daily.

The vials containing the cuttings were placed in numbered racks each holding ten or twenty vials. It was found possible to test as many as twenty-five racks (250 solutions) at one time. After a standard arbitrary time of two weeks under any particular test, the cuttings were dissected under the binocular microscope and inspected for floral primordia. As shown in table 16, this period is approximately twice as long as is required for the initiation of detectable floral primordia by similar cuttings subjected to short photoperiod.

A number of the substances known to possess activity as plant growth factors were investigated by the cutting test for possible activity in floral initiation. Thus vitamins B₁, B₂, B₆, ascorbic acid, pantothenic acid, nicotinic acid, inositol, indoleacetic acid, theelin, and theelol were used over wide ranges of concentrations. Yeast extract, which contains several of these growth factors, was also used. All these substances were found to be completely without activity in initiating floral primordia on *Xanthium* cuttings. Vitamin B₁, theelin, and yeast extract were also supplied in the nutrient solution given to *Xanthium* plants in sand culture and maintained under long photoperiod. Despite the great promotive effects of these materials on growth, the plants remained strictly vegetative. In another experiment vitamins B₁ and B₆, pantothenic acid, and nicotinic acid were supplied to vegetative *Xanthium* plants through funnels of appropriate size attached to the stump of the decapitated epicotyl. These plants also remained vegetative. It seems probable that the substance responsible for floral initiation is not identical with any of these known plant growth factors.

Vitamin B₁, ascorbic acid, theelin, theelol, and yeast extract were also investigated for possible promotive effects upon the development of chrysanthemum flowers. Plants of a late flowering clone were subjected to eight short photoperiods, after which they were found to have microscopically detectable floral primordia. They were then returned to long photoperiod, transplanted to sand, and supplied with nutrient solution containing various concentrations of the above growth factors. No effect upon the continued development of the microscopic floral primordia into macroscopic flowers was found in any case.

Since the work of GARNER and others (8) has shown that the initiation of the flowering state by appropriate photoperiod is associated with changes of acidity, a number of the common organic acids found in plants were tested, each over a wide range of concentrations, for possible effects on floral initiation on *Xanthium* cuttings. Succinic acid, malic acid, tartaric acid, citric acid, fumaric acid, oxalic acid, and maleic acid were found to be without effect, as were also the amino acids arginine, lysine, and glutamic acid.

Two hundred and forty-six different kinds of extracts and extract fractions were made during the course of attempts to extract the floral initiation substance from leaves. Extraction with several solvents, such as water, ethyl alcohol, ether, chloroform, acetone, benzene, and pyridine, was employed, and both fresh and oven-dried leaves were used. Considerable precaution was exercised in the treatment of these extracts. All concentrations and evaporations were done in an atmosphere of N₂ under diminished pressure and below 60° C. in general, to decrease possible oxidation of the active material. Each extract was tested in several concentrations, so that more than 1150 solutions were tested, and approximately 4500 cuttings dissected. About half of the extracts were of leaves from *Xanthium* plants which had been subjected to short photoperiod; but extracts of other short day plants, as well as of indeterminate and long day plants, all in the flowering state, were prepared. None of these extracts showed any activity in bringing about the initiation of floral primordia on *Xanthium* cuttings when tested in the manner described.

There are indications, however, that if the vegetative cuttings are

treated by slight modifications of the procedure here outlined, they may respond with the initiation of floral primordia to the application of a simple water extract of leaves from induced *Xanthium* plants.

IX. Discussion

The response of *Xanthium* to photoperiod appears to differ in some respects from that of certain other photoperiodically sensitive plants which have been investigated. The strict localization of the response which has been reported for leafy plants of cosmos (7), chrysanthemum (2), and of other plants, is not found in leafy plants of *Xanthium*. In the species other than *Xanthium*, leaves of shoots subjected to conditions of long photoperiod prevent the initiation of floral primordia upon such shoots, even though other portions of the same plant are subjected to conditions of short photoperiod. Localization of this kind can be obtained with *Xanthium* provided only mature, fully expanded leaves are allowed to remain on a receptor shoot subjected to long photoperiod. Mature leaves of *Xanthium* thus behave in this respect in a manner similar to the leaves of some of the other plants so far investigated. Young expanding leaves of *Xanthium*, on the contrary, appear to exert a promotive effect on floral initiation and flower development in receptor branches.

Xanthium differs from soy bean (1), chrysanthemum, and cosmos (7) in that exposure of a *Xanthium* plant to a number of short photoperiods sufficient to result in the initiation of floral primordia also results in the development of these primordia into mature flowers and fruits, despite subsequent maintenance under conditions of long photoperiod. Plants of the other species under similar conditions initiate floral primordia but these frequently do not develop into mature flowers or fruits unless exposed to a still longer induction period.

Xanthium plants initiate floral primordia after exposure to one long dark period at 21° C., irrespective of whether this dark period is immediately preceded by a long or a short photoperiod at a relatively low or relatively high temperature. On the other hand, soy bean (1) must be subjected to at least two long dark periods separated by one photoperiod, for the initiation of floral primordia. Evidently the initiation of floral primordia by *Xanthium* is the result of processes

taking place during the dark period. Whether or not other species or varieties of plants could be induced to initiate floral primordia during a single dark period under certain conditions of temperature or humidity remains to be determined. It may be that in some plants floral initiation is conditioned by processes taking place during the photoperiod as well as during the dark period.

On the basis of data presented in the foregoing experiments, the behavior of most short day plants and probably also of long day plants and many others can most readily be attributed to the presence of a floral initiating substance whose genesis is probably in the fully expanded leaves when directly exposed to appropriate photoperiods or dark periods. Such substance may be transported throughout the plant.

X. Summary

1. A description is given of a simple method whereby one portion of a plant may be subjected to one photoperiod while another portion of the same plant is being subjected to another photoperiod.

2. Floral initiation in *Xanthium pennsylvanicum* results if plants are subjected to photoperiods shorter than 15 hours with accompanying dark periods of longer than 8 hours. If *Xanthium* plants are subjected continuously to photoperiods longer than 16 hours with accompanying dark periods shorter than 8 hours they remain strictly vegetative.

3. The initial effect of the photoperiodic stimulus is perceived by the leaves which are subjected to short photoperiod. However, this stimulus, resulting in floral initiation, may be transported from these leaves to other portions of the same plant which are maintained under conditions of long photoperiod and may also move across a diffusion contact from a plant subjected to short photoperiod to a plant subjected to long photoperiod. The stimulus to floral initiation may therefore be attributed to a substance or substances manufactured in leaves subjected to short photoperiod.

4. The response of *Xanthium* to photoperiod is primarily a response to length of dark period rather than to duration of photoperiod. Thus reactions resulting in the formation of floral initiation

substances may take place during the dark period. These reactions are adversely affected by light and by low temperature.

5. Fully expanded leaves on receptor branches subjected to long photoperiod may exert some influence inhibitory to floral initiation; under similar circumstances young expanding leaves exert a promotive effect on floral initiation and flower development.

6. In *Xanthium* the development of mature flowers and fruits from floral primordia is also promoted by a substance or substances formed in portions of the plant which are exposed to short photoperiod and which may move across a diffusion contact. Whether or not this substance or substances is identical with the floral initiation substance has not as yet been determined.

7. A portion of a plant maintained under long photoperiod may be influenced by a portion of the same plant subjected to short photoperiods in such a way that it may behave as though it has been photoperiodically induced by direct exposure to short photoperiod. Flowers and fruits continue to develop on such portions of a branch which has never itself been subjected to short photoperiods.

8. Evidence is presented that the floral initiation substance is not identical with any of the following known plant growth factors: vitamins B₁, B₂, and B₆, ascorbic acid, nicotinic acid, pantothenic acid, theelin, theclol, inositol, or indoleacetic acid.

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CURRENT LITERATURE

Vergleichende Morphologie der niederen Pflanzen. Erster Teil: Formbildung. By BRUNO SCHUSSNIG. Berlin: Gebrüder Borntraeger, 1938. Pp. viii+382. Figs. 470. Rm. 36 unbound; Rm. 38 bound.

This comparative morphology of the lower plants considers the algae and fungi as a whole, and takes them up topic by topic instead of group by group. The present volume covers the vegetative structures; the second part will cover the reproductive organs, alternation of generations, sexuality, heredity, and evolution.

The book is divided into four sections, and in each of them much more attention is given to the algae than to the fungi. The first section, the comparative morphology of the cell, discusses the cytoplasm, the nucleus, the locomotor apparatus, the plastids, the vacuolar system, and the cell membrane. In each chapter there is a comprehensive account of the structures concerned for both the algae and fungi. In many respects this is the most satisfactory portion of the book, possibly because the general subject is one well adapted to the comparative viewpoint from which the whole book is written. However, one wishes that there were more direct references to the bibliography, which will appear in the following volume.

The second section has two chapters devoted to cell division, and one to a consideration of the cell as a whole. The third section, covering the comparative organography of the lower plants, sets up a series of types of plant body and proposes an elaborate new nomenclature for the various types. In it the plant bodies are analyzed according to their fundamental organization and are placed in three major categories. The first of these, the Nematoblasts, includes all those in which the plant body is resolvable to a single filament. The second, the Siphonoblasts, includes all those with a siphonaceous type of plant body. The third group, the Syngamata, includes all in which the plant body consists of several or many filaments. It is the equivalent of OLTMANN's "fountain type" of thallus. In the opinion of the reviewer this is the least satisfactory section of the book. One objection is the introduction of a new and elaborate terminology; a second and more important objection is the question of whether, when taken as a whole, the thallus construction among algae and fungi is a matter of fundamental significance. In certain respects the author's rather artificial classification of thalli of algae and fungi is reminiscent of the old herbalists' segregation of vascular plants into herbs, shrubs, and trees.

The concluding section is devoted to comparative anatomy, and discusses the

cortical system, the storage system, the medullary system, and the mechanical system of the thallus.

The problem of appraising the value of this book is an exceedingly difficult one. The first section will certainly be of service to those seeking information concerning the cytology of primitive plants. So far as the remainder of the book is concerned, one can say that it can be read to advantage only by botanists well acquainted with the algae.—G. M. SMITH.

The genus Septobasidium. By JOHN N. COUCH. Chapel Hill, North Carolina: University of North Carolina Press, 1938. Pp. ix+480. Illustrated. \$5.00.

The acrid controversy concerning the dual nature of lichens is now recalled only as one of the curiosities of botanical history. So thoroughly entrenched has the newer view become that the recent demonstration of an even more striking relationship between a group of fungi and certain scale insects has failed to attract the attention it deserves. There are far fewer species of *Septobasidium* than of lichens, to be sure, and their distribution is more restricted, but they are both more abundant and more widespread in tropical and temperate regions than has generally been realized, and the relationship with the insect hosts is much more highly specialized than that between a lichen fungus and its associated alga. This relationship, first cautiously suggested by VON HÖHNEL and LITSCHAUER in 1907, has been investigated by a limited number of students, among whom the author of the present volume has been outstanding. He has now embodied the results of these studies in a beautifully illustrated work which summarizes in completely adequate fashion all that has been learned up to the present time concerning these highly significant forms.

The account of the symbiotic relationship between the fungus and the insect is necessarily based upon detailed observation and experiment involving only a limited number of species, but the evidence is overwhelming that the conclusions drawn are applicable, in all essential particulars, to the entire group. It is shown that the fungus is absolutely dependent upon parasitized insects for its nutrition, while at the same time it furnishes shelter and protection for the unparasitized individuals, without which these are scarcely able to maintain themselves. Dissemination of the fungus from host to host is apparently by the passage of both parasitized and unparasitized insects, presumably by crawling where the branches are in contact, or otherwise perhaps on the feet of birds. Woody plants bearing *Septobasidium* may be severely injured. The injury, however, is primarily due to the scale insects. The fungus shelters the insects, protecting them even against the effects of strong sprays, and is thus the effective factor in bringing about the damage.

On the basis of the symbiotic relationship, COUCH suggests that the Septobasidiaceae be raised to the rank of an order, coordinate with the Auriculariales, Uredinales, and Ustilaginales among the Heterobasidiomycetes possessing transversely septate basidia. He retains all species in the single genus *Septo-*

basidium, justifiably believing that this name should be conserved against the earlier and technically valid name *Glenospora*.

Of the 163 species, 5 varieties, and 2 hybrids described, all but a very few are illustrated, for the most part both by reproductions of photographs showing the habit and by figures of microscopic details. The account of one, at least, of the hybrids is far more convincing than references to hybrid fungi are wont to be. Fifty-six species, including four to which old herbarium names are affixed, are described for the first time. Technically they are not validly published, since Latin diagnoses are not supplied. But even those who strongly favor the Latin requirement can scarcely fail to recognize species so carefully described and illustrated.

It is noteworthy that a larger number of known species occurs in southeastern United States than in any comparable area. There can be little doubt that this is because most mycologists are inclined to pass over species of *Septobasidium* as sterile lichens, which many of them strikingly resemble. With more discriminate collecting, particularly in the tropics, it may confidently be expected that the number of species will be greatly increased.

In keeping with its character, the book has been meticulously edited and is singularly free from typographical errors.—G. W. MARTIN.

Micropedology. By WALTER L. KUBIĚNA. Ames, Iowa: Collegiate Press, 1938. Pp. xvi + 243. Illustrated. \$3.00.

The book is a summary of some of the methods used and the results obtained by the author and collaborators during the past several years in a direct microscopic study of the soil. Fully half of the volume is devoted to the techniques employed. The use and development of incident-light microscopes, micromanipulations, soil preparation methods, and microchemical and optical methods of analysis are discussed. While these methods are primarily designed to aid in the study of mineral components, there are several worthwhile developments which should find application in biology.

A further large section of the book is devoted to the study of soil fabrics, by which the author means the "arrangements of constituents in the soil and their role in relation to each other." These studies, of more interest to the geologist and pedologist, serve as a background for the discussion of soil biology. The observations reported clearly demonstrate the influence of the microclimatic conditions upon not only the ecology, but even the size and character of the different soil inhabitants. These microhabitats are in a sense independent units, except for interrelations which make each habitat part of the same biological system. Application of the direct microscopic method to the characteristics of the microhabitats, to decomposition of organic matter and humus formation, and to the ecology of the soil flora and fauna is discussed. The method developed does not yet enable the direct observation *in situ* of bacteria, but KUBIĚNA feels that this ideal may be soon accomplished. At present it is necessary to supplement

studies with the Rossi-Cholodny and Winogradsky technique. A brief survey of some of the preliminary results emphasizes the great potentialities of the method for a finer understanding of the role of both the plant and the micro-organism in the biology of the soil.—W. W. UMBRETT.

Die Chromatographische Adsorptionsmethode. By L. ZECHMEISTER and L. V. CHOLNOKY. Vienna: Universitat Pecs. Julius Springer, 1937. Pp. 231. Illustrated. Rm. 14.40.

The rapidly increasing usefulness of chromatographic adsorption technique in the separation and purification of compounds makes this volume very welcome, especially to biological chemists. The authors quote in their preface "Jeder wissenschaftliche Fortschritt ist ein Fortschritt der Methode." In this discussion of adsorption methods, other workers should find many observations which will aid them in related problems.

The book is divided into a general discussion of the adsorption method and a more specialized section in which its application to specific mixtures of naturally occurring substances is discussed in detail. In the first section the relationship between order of adsorption on a chromatogram and constitution of compounds is discussed, especially the case of conjugated systems. The carotenoid pigments and derivatives provide the most familiar cases.

The second section includes detailed discussions of specific experiments and many literature references. Some groups discussed are: chlorophyll, carotenoids, flavins, anthocyanes, certain colorless compounds, plant and animal poisons, alkaloids, enzymes, vitamins, and hormones. An extensive table of summarized adsorption data on various carotenoids is presented.

The volume closes with a number of excellent photographs, in black and white, of adsorption columns. The entire book is full of illustrations, diagrams, and discussions of experimental detail, which highly recommend it, especially to those starting work with plant pigments.—F. P. ZSCHEILE.

Chromosome Number Relationship in the Leguminosae. By HAROLD A. SENN. *Bibliographia Genetica*, Vol. XII. Pp. 175-336. 1938.

This contribution represents a preliminary survey of the chromosome number relationships in the Leguminosae, including some 233 chromosome number determinations made by the author on 106 species of 33 genera, together with a list of the known chromosome numbers in 436 species of 74 genera in the family. The following haploid numbers have been determined: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30, 32, 40, 48, 65. The basic number for the Papilionatae SENN considers to be probably 8.

Certain taxonomic changes are corroborated on the basis of the cytological findings, and the need for other changes is indicated. A hypothetical phylogenetic chart of the family, based on chromosome number relationships, and to a limited extent on morphological relationships, is presented. A primitive

8-chromosome papilionate stock seems to have given rise along at least four distinct lines to the present day Papilionatae. The 7-chromosome Viciae probably arose as hypoploids from the primitive 8-chromosome papilionate stock, and the 11-chromosome Phaseoleae may have arisen through hybridization of a tetraploid of the 7-chromosome Viciae line with a diploid of the 8 line.

Polyploidy is found to be low in the Leguminosae, only 23 per cent being found to be polyploids or derived from polyploids. The relation of polyploidy to geographic distribution is reviewed and discussed, and doubt is cast on the validity of the view that perennial and woody conditions are primitive. In general, the numerical data on chromosome numbers of the Leguminosae support MUNTZING'S conclusions of 1936 that higher numbers are associated with the perennial condition and indicate that in the Leguminosae, at least, some of the woody perennial species may have originated from herbaceous annual species.—J. M. BEAL.

Trees of Northeastern United States; Native and Naturalized. By H. P. BROWN. Boston: Christopher Publishing House, 1938. Pp. 490. Illustrated. \$3.00.

This book is the result of the revision and enlargement of Technical Publication 15 of the New York State College of Forestry, Syracuse, N. Y., which appeared in 1921 under the title of *Trees of New York State; Native and Naturalized*. The book, written by an experienced teacher of dendrology and wood anatomy, is for teachers, botanists, foresters, and others who may wish seriously to study trees. It is scientific but non-technical.

The principles upon which the taxonomic classification of plants is based and a brief but clearly stated review of the morphology of woody plants are included. Readily usable separate keys, based on leaf, fruit, and twig characters, precede plates with legends and tabulated descriptive information for each of the 152 species figured. The illustrations are excellently drawn and are unusually complete in showing leaves, flowers, fruit, and winter twigs. Each species is carefully described under the following headings: habit, leaves, flowers, fruit, winter characters, habitat, range, and uses. The many excellent qualities of the book warrant its continued use for at least another twenty years by all who may need a tree manual for the northeastern United States.—C. F. KORSTIAN.

The Structure of Economic Plants. By HERMAN E. HAYWARD. New York: Macmillan Co., 1938. Pp. x+674. Figs. 340. \$4.90.

During the last number of years there has been an increasing emphasis on the study and recording of the developmental aspects of plant anatomy in addition to the purely descriptive and phylogenetic phases. The former is of particular importance to all who must deal with the general problem of plant production, physiologists and pathologists. Studies of function are rendered far more direct and effective through a knowledge of structure. To know the pattern and course of development of a given plant species or variety is of utmost importance

to botanists and plant husbandmen alike. Often such patterns may be shifted through wide limits, both quantitatively and qualitatively, dependent upon the ranges of environment imposed. In applied terms this means that the yield and quality of the crop produced are in large measure the tangible results of a specific agricultural practice. In recognition of this fact, the author has laid particular stress on those parts of any given species which are of greatest economic importance, but has adequately treated the details of the entire plant as well.

The book is organized in two parts, the first dealing with general principles of plant anatomy and the second with the structure of sixteen plants of economic importance.

While occupying only one-sixth of the space, the chapters covering cells and tissues, the anatomy of the root, shoot, flower, and fruits, not only furnish an excellent introduction to general plant anatomy, but also acquaint the reader with the nomenclature which is to be used.

The author has not refrained from discussing controversial points and presenting the opposing views of critical investigators with reference to the morphological interpretation of certain tissue systems, flower and fruit structures. Such presentation, however, at no time overshadows a precise recording of tissue origins and detailed description of plant organs. The book brings together in one place for the first time the type of material long needed by workers in the broad field of plant industry. It is, however, far from a simple compilation of scattered data. Much of it is based on original investigations by the author, his co-workers and students, so that the work represents in its own right a distinct contribution to anatomical knowledge. A very considerable proportion of the illustrations are original and were prepared under the author's direct supervision. Many others are used for the first time outside the original publications. This liberal use of new material is particularly refreshing.

Part II deals in detail with sixteen crop plants—corn, wheat, onion, hemp, beet, radish, alfalfa, pea, flax, cotton, celery, sweet potato, white potato, tomato, squash, and lettuce. All are treated at length in a clear, direct style. This selection was determined by "the economic importance of the plant, its suitability as a representative of the family to which it belongs, and the intricacy of its anatomical and morphological detail."

The author states that he contemplates a companion volume dealing with important fruit crops. If it is as admirably done as the present one it will be enthusiastically received by all who work with plants.—J. H. GOURLEY.

Useful Trees and Shrubs. By FLORENCE BELL ROBINSON. Champaign, Illinois: The Garrard Press, 1938. \$4.50.

This new venture in publishing consists of almost 500 4×6" cards, each treating one species of ornamental tree or shrub. Cards of different colors are employed for the five groups of 206 deciduous shrubs, 148 deciduous trees, 62

conifers, 39 broad leaved evergreens, and 36 vines included. The data are necessarily of a general nature, expressing average conditions in the north-eastern states and Canada. Statements concerning native habitat, hardiness, form and size, color at different seasons, root system, habitat preferences, periods of foliage, flowers and fruit, cleanliness, response to pruning, aspect, and value are included. Space is provided for sketches of summer and winter condition. Local variations may be noted on the back of the card. Nomenclature follows Standardized Plant Names. This file should be useful to students, amateurs, and professionals in the field of landscape architecture, and to nurserymen.—C. E. OLMSTED.

The Nation's Forests. By WILLIAM ATIHERTON DU PUY. New York: Macmillan Co., 1938. Pp. xii+264. Illustrated. \$3.00.

This book is a popular and well written elementary account of the nation's forests, their early exploitation, the creation of the National Forests, and the principles evolved for their management. The latter includes chapters on cutting practices, planting, sustained yield, erosion control, fire prevention, recreation, wild life, range management, and wilderness areas. Chapters on wood products and state and private forestry conclude the book. It is copiously and beautifully illustrated. More than half the pages are full-page halftones, the pictures largely obtained from the files of the U.S. Forest Service, which throughout receives its full measure of praise.—C. E. OLMSTED.

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TRANSLOCATION OF CARBOHYDRATES IN THE CUTHBERT RASPBERRY¹

CHARLES J. ENGARD

(WITH FOUR FIGURES)

Introduction

Many investigations have been conducted on the raspberry plant, especially in regard to the economically important aspects such as resistance to freezing, pruning, fertilizing, etc., but there has been little study relative to the forms and movements of the chief organic substances. DU SABLON (15) included the raspberry in his study of the reserve materials of trees. BRIERLEY (2, 3) studied senescence in the red raspberry cane, emphasizing cambial decline and phloem disintegration, and the responses to pruning. BRIERLEY and LANDON (4) presented evidence on the downward movement of carbohydrates in the fruiting canes. BENNETT (1) demonstrated that the movement of the leaf-curl virus of raspberries takes place in the phloem and in the direction of the major transport of food. Reviews of the literature in translocation have been published by CLEMENTS (8), CURTIS (14), and more recently by MASON and PHILLIS (22) and CRAFTS (13).

Material and methods

The Cuthbert raspberry is a hybrid derived from *Rubus idaeus* L. and *R. strigosus* Michx., the latter considered by HEDRICK (17) as a

¹ Contribution no. 57 from the Botany Department of the State College of Washington.

subspecies of *R. idaeus*. It produces tall erect canes which attain their full height and most of their diameter growth within the first growing season. Fruiting occurs in the second season on laterals developed from over-wintered buds. At the height of the harvest season disintegration of the phloem occurs at the base and in the apical region of the cane. BRIERLEY (2) thinks the former is associated with the senile condition of the cambium, and the latter caused by a disturbance in water conduction.

Because the various canes are relatively uniform in size, length, and diameter, the Cuthbert raspberry was chosen for experimental material. It was at first intended to include second year (fruiting) canes as well as first year ones, thus making a complete study up to and beyond fruiting; but during the winter previous to the beginning of this study all canes were winter killed, and none which could be used during the following growing season could be found in the locality.

The experimental plants were mosaic-free stock grown by the Department of Horticulture at the State College of Washington.² They were grown on a uniform Palouse silt loam soil with ample moisture for growth, and on a southwest slope. The rainfall of this region comes mostly during the winter and spring; after May gradual drying of the soil occurs up to the end of September, when there are occasional showers.

In this study double ringing was employed. By ringing at a point 15 cm. and again at a point 30 cm. above the ground, a segment of bark 15 cm. in length was isolated. The strips of bark removed were less than 1 cm. wide, completely girdling the stem. The lower ring prevents upward movement in the bark of certain substances from the roots and other normal canes growing therefrom, and the upper ring prevents downward movement of materials mobile in the bark into the isolated segment below.

On May 15, 1936, when the new shoots were about 60 cm. high, some were ringed, some tagged for controls, and others were taken into the laboratory and prepared for subsequent analysis. The plants harvested at the time of ringing on May 15 served as checks

² Thanks are due to the Department of Horticulture, State College of Washington, for the raspberry plants used throughout this investigation.

for the June series. Ringed canes and the controls tagged on May 15 were harvested on June 23, and another set of canes were ringed and others tagged for additional controls on this date. These were harvested on July 16 and constitute the July series. On July 16 canes were ringed, and others tagged for controls; these were harvested on August 9, as the August series. Control plants for any series (except May) were those which were tagged but not ringed in the immediately preceding month. Twenty-four canes were used in each treatment, twelve for nitrogen and twelve for carbohydrate analyses.

At each collection the canes were severed at the ground level. Each cane was cut into 15 cm. segments, measuring from the base, so that the sample contained the equivalent segment of twelve canes. The leaves of each segment were cut off at the junction of petiole and stem and were analyzed in aggregates of twelve equivalent segments. The stem samples were analyzed separately. The top segment (or portion thereof) and its young leaves were analyzed as one sample. For these various samples the following symbols have been adopted: (1) Each 15 cm. segment of stem, numbering from the base to the top, is designated as 1st, 2st, 3st, etc. Thus in the double ringed canes, segment 1st is that below the lower ring; segment 2st is that between the rings; and segment 3st is that above the upper ring. (2) The several leaves diverging from each segment are symbolized as 3L, 4L, 5L, and 6L, these being from segments 3st, 4st, 5st, and 6st respectively. The leaves usually drop from the first 30 cm. of the raspberry stem, so that in these experiments the leaves were cut from the first two segments at each treatment. (3) The uppermost segment (or portion thereof) with leaves too small to constitute a separate sample, is designated as tops.

The segmentation was done as rapidly as possible, and all samples were immediately weighed and autoclaved at 15 lb. pressure for 15 minutes. After drying in a ventilated oven heated to 60° C., the material for carbohydrate determinations was ground to 40-mesh fineness in a power mill.

MOISTURE.—The moisture content of the carbohydrate samples was determined by drying them for a week in an oven at 60° C. and then weighing.

CARBOHYDRATES.—The procedure was essentially that described by CLEMENTS (6), with the exception that the term “acid hydrolyzable carbohydrates” has been substituted for “hemicellulose.” This group was taken out in two fractions (11), one after three hours of hydrolysis on the steam bath and the second after twelve hours. The first group, designated as “acid hydrolyzable A,” is presumably made up of polysaccharides of high molecular weight which are held more or less loosely to the cell wall or within the protoplast. The second group, “acid hydrolyzable B,” is made up of polysaccharides which are more stable and which are possibly part of the secondary thickening of the cell wall.

TABLE 1
DISTRIBUTION OF CARBOHYDRATES IN NORMAL PLANTS
MAY 15 (BEGINNING CONTROLS)

CANF SEGMENT	TOTAL CARBOHYDRATE		REDUCING SUGARS		SUCROSE		STARCH		TOTAL ACID HYDROLYZABLE	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)
1st.....	5.80	27.70	1.40	06.0	0.06	0.24	0.18	0.77	4.16	17.70
2st.....	4.98	31.13	1.67	10.4	0.07	0.46	0.23	1.44	2.91	18.18
3st.....	2.72	33.18	1.20	14.7	0.07	0.83	0.18	2.20	1.27	15.40
Tops.....	2.53	33.72	1.04	13.9	0.05	0.71	0.18	2.40	1.26	16.8c
3l.....	12.20	36.40	4.94	14.1	0.05	0.15	0.07	0.21	5.71	17.05

EXPRESSION OF RESULTS.—The basis upon which to calculate and report analytical results is a prevalent controversy. In the light of considerations to be reported elsewhere (16), the following procedure is used in expressing results: (1) When it is especially desirable, absolute changes in a segment are expressed as the weight difference in grams per sample (equivalent segments of twelve canes = a sample). (2) Percentage concentrations are used in most cases, such as those dealing with gradients. The substances of the control plants are expressed as percentages of their residual dry weight. The substances of the treated plants are expressed as percentages of the residual dry weight of the corresponding controls.

Investigation

SEASONAL DISTRIBUTION OF CARBOHYDRATES IN NORMAL PLANTS

TOTAL CARBOHYDRATES.—The percentage of total carbohydrates, which includes the material hydrolyzable in 2.5 per cent acid in fifteen hours, is fairly high in the stem and leaves of the plants harvested in May (table 1). It drops during June, the period of most

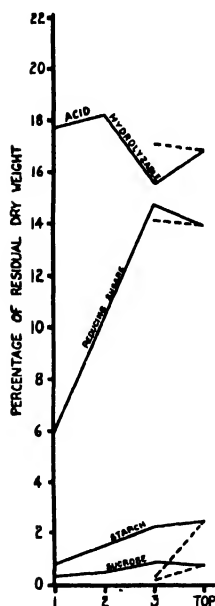


FIG. 1

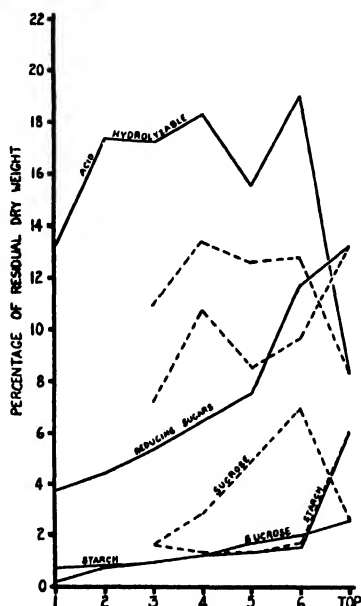


FIG. 2

FIGS. 1, 2.—Fig. 1 (left), distribution of carbohydrates in normal cane segments, May 15. Dotted lines represent leaf samples corresponding to stem segments. Cane segments, numbering from base of stem to top, are indicated along abscissa. Fig. 2 (right), distribution of carbohydrates in normal cane segments, June 23.

rapid growth, and attains a maximum in August. The drop during the period of rapid growth corresponds to that observed by DU SABLON (15) in the raspberry. He attributed the early spring high to a migration of carbohydrates from the roots into the stems. He obtained a maximum of total carbohydrates, as percentages of the dry weight, in October. The concentrations of total carbohydrates are slightly lower in the lower segments than in the upper in the May

and June collections. This ascending order practically disappears during July and August. All fractions of the total carbohydrates for the normal plants are plotted in figures 1 to 4.

ACID HYDROLYZABLE CARBOHYDRATES.—This group of substances, the largest fraction of the total carbohydrates, made up of heterogeneous cellulosic compounds—polysaccharides of high molecular

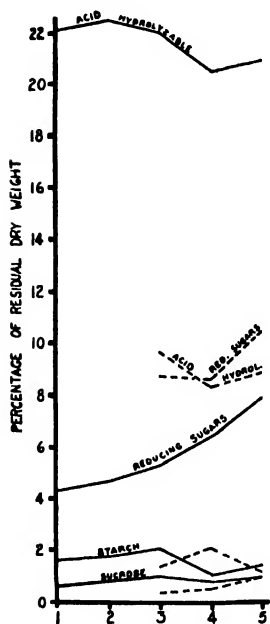


FIG. 3

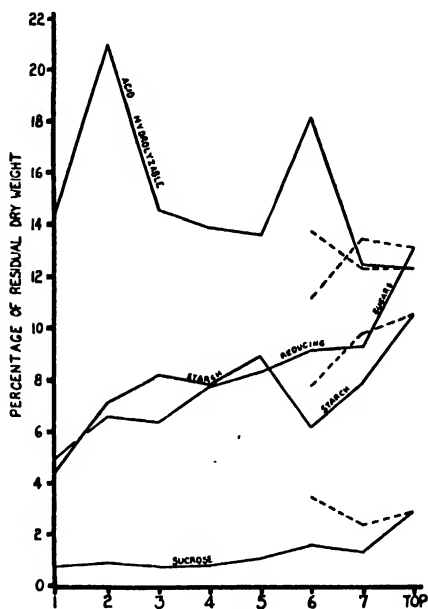


FIG. 4

FIGS. 3, 4.—Fig. 3 (left), distribution of carbohydrates in normal cane segments, July 16. Fig. 4 (right), same, August 9.

weight and their derivatives—have varying degrees of stability upon hydrolysis. For this reason, hydrolysis for three hours and then for an additional twelve hours yielded very erratic results. For example, the concentration of the former or A fraction varied from 1 per cent in one segment to 10 per cent in the neighboring segment, whereas the second or B fraction would be the reverse. The total of the two fractions in any one segment, however, was comparable with that of the other segments. The idiosyncrasies of the less stable “hemicelluloses” disappeared upon continued hydrolysis, although some in-

consistency still remained. For this reason the individual A and B fractions of the acid hydrolyzable substances were not graphed nor tabulated. Separate hydrolysis, however, has the advantage of largely preventing the destruction of that portion of the hydrolysate which is produced early in the boiling process.

With the exception of the May series, the tops are lowest in concentration of these substances. The second segment is always considerably higher in concentration than the first, and with the exception of the June series the concentration in the second segment is the highest of all parts of the plant. This may be owing to its location just below the foliage region, for, as BRIERLEY (2) has shown for fruiting canes, cambial activity is greatest in the region below a vigorous lateral or foliage, and is usually absent in the base of the cane. In 90 per cent of the canes measured by BRIERLEY (2) he found no measurable increase in diameter at the base of old canes. There may be some relationship between the erratic activity of the raspberry cambium in various regions of the stem and the erratic results obtained for the acid hydrolyzable material along the stem, but the results are probably caused by the variable responses of the substances to hydrolysis.

In all but the May series the amount of acid hydrolyzable material is lower in the leaves than in the stem segments. The increase of the acid hydrolyzable fraction in the tops of the May series is accompanied by a drop in the reducing sugars. The graph of this drop (fig. 1) from 3st to tops, and the leaves of 3st to tops, is nearly a mirror image of the graph of the acid hydrolyzable substances in the same region. The amounts of reducing sugars in this series are higher than in any other collection, but starch and sucrose are lower. The highest concentration of sugars is nearly 700 per cent higher than that of starch. There seems to be a close relationship in the May series between the high concentrations of reducing sugars and the acid hydrolyzable carbohydrates. This indicates that not only are the "hemicelluloses" a form of food reserve, but they may be an even more immediate storage form than starch. Thus it is concluded with CLEMENTS (6) that, "the vacuolar sugars may be so concentrated as to favor the formation of some reserve which is entirely removed from the solution," and that, "under favorable conditions

for rapid carbohydrate formation they [the hemicelluloses] seem to act as temporary reserves in the leaves."

REDUCING SUGARS.—The highest concentrations of reducing sugars are in the May series, drop rapidly in the June series, from whence they increase only slightly in July and August. The basal segment is always lowest in reducing sugars, with concentrations increasing toward the top. The leaves diverging from the subterminal segment have less sugars than that segment, except in the August series. Lower leaves are always higher in concentration of sugars than their corresponding segment. The large amount of reducing sugars in all parts of the plant seems to indicate that it is an important temporary storage form.

STARCH.—Starch is never abundant, and does not rise above 6 per cent except in the August series, where it essentially parallels the reducing sugar curve. Apparently it is not an important reserve in the first year canes of raspberry. The starch content of leaves is lower than that of the corresponding stem segments in May, is equivalent in June, slightly above in July, and definitely higher in August. The gradient is not steep and the basal segment is lowest in starch content.

SUCROSE.—Sucrose is present at all times in all parts of the plant, but its concentration is low, rising above 2 per cent only in June and in August. It becomes highly concentrated in the leaves of the June series, reaching a maximum in the uppermost and grading downward to the lowermost leaves.

The very low concentration of this sugar indicates that it is not an important one, either for direct use as an energy source or for temporary storage.³ Its presence throughout the plant suggests that it has a definite role in metabolism, probably of translocation. That it does not result as a condensation product when reducing sugars attain a certain maximum concentration, such as CLEMENTS (6) found in his work on the sunflower, soy bean, and potato, is indicated by an entire lack of relationship between the varying concentrations of reducing sugars and the little-fluctuating amounts of sucrose. There seems to be no relationship with moisture content, as LEONARD (18)

³ Microchemical data to be presented in a later paper indicate that sucrose is limited in its distribution, essentially to phloem, making it inconceivable that sucrose functions as an important source of energy.

found in the sunflower. The very small amounts of sucrose in the stem indicate that, if it is a translocatory form, it is confined to a very small part of the total mass of stem, presumably the phloem. The actual concentrations in this tissue are thus greatly reduced by the total mass of the stem. The slight gradient which appears in figures 1 to 4 is insignificant and is probably nonexistent. It is the result of dividing the grams of sucrose by residual dry weights which are somewhat greater in the lower than in the upper portions of the plant.

EFFECT OF RINGING

The effects of double ringing on the sugars in the treated plants are compared with the untreated plants in tables 2 to 4. The usual accumulation of carbohydrates is found in the parts above the rings owing to the prevention of movements into regions below the ring. According to MASON and MASKELL (20), ringing is noticeably effective in the interruption of the downward movement of carbohydrates within two hours. In all series there is a great increase of total carbohydrates above the rings over the normal concentrations. Accompanying this increase is an even greater decrease of total carbohydrates between and below the rings.

Increases or decreases in the ringed canes, expressed in whole numbers as percentages of the normal concentrations, are given for the segments below the rings (1st), between the rings (2st), and just above the rings (3st) in table 5. It is evident that all carbohydrate substances, except starch in the June series, make large percentage increases in the segment above the upper ring. The largest increase was in the amount of sucrose. The percentage increase of reducing sugars was only half that made by sucrose.

All fractions decrease greatly between the rings. Sucrose again was affected most, there being a 75 per cent reduction in June, and a 100 per cent reduction in July and August. The reducing sugars showed not so great an apparent decrease, but much of that present at the time of analysis was undoubtedly derived from starch and acid hydrolyzable carbohydrates, as well as from the residual polysaccharides (table 7). The data in tables 5 and 7 indicate that carbohydrates other than reducing sugars were 43 to 100 per cent lower than normal.

TABLE 2
SEASONAL CHANGES IN TOTAL CARBOHYDRATES AND
REDUCING SUGARS IN NORMAL AND RINGED PLANTS

CANE SEGMENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)
TOTAL CARBOHYDRATES												
1st	4.00	17.75	1.67	7.44	6.60	28.41	3.82	16.47	8.20	35.68	5.10	22.15
2st.	3.70	22.98	1.13	6.99	5.10	29.48	2.81	16.23	6.40	35.55	2.28	12.67
3st.	3.30	23.73	5.12	36.85	4.40	30.55	5.54	38.40	4.80	29.45	7.73	47.40
4st.	3.10	28.43	4.27	39.25	2.80	28.90	4.52	46.70	3.90	30.00	7.02	54.00
5st.	2.10	26.25	3.03	37.85	2.50	31.28	4.14	51.75	3.10	31.60	4.33	44.30
6st.	1.40	34.15	1.93	47.10	2.70	34.60	4.01	51.40
7st.	1.90	30.15	2.79	44.30
Top.	3.80	29.45	3.55	27.50	5.90	38.80	6.05	45.70
7L.	6.10	37.45	13.85	85.00
6L.	5.20	30.75	5.45	32.20	6.30	36.22	15.44	88.80
5L.	5.60	27.45	5.87	28.80	5.80	29.00	6.10	30.50
4L.	5.40	28.78	6.10	31.95	4.20	23.60	5.70	32.20
3L.	3.50	21.07	5.17	31.15	4.60	22.45
REDUCING SUGARS												
1st.	0.85	3.78	0.37	1.64	0.99	4.27	1.28	5.52	1.14	4.96	0.93	4.04
2st.	0.71	4.40	0.23	1.45	0.82	4.64	0.61	3.53	1.18	6.55	0.49	2.72
3st.	0.74	5.35	1.52	10.90	0.76	5.28	1.24	8.61	1.03	6.30	2.75	16.87
4st.	0.70	6.43	1.24	11.40	0.62	6.39	0.95	9.80	1.01	7.77	2.06	15.85
5st.	0.60	7.50	1.14	14.20	0.63	7.88	1.05	13.14	0.81	8.27	1.50	15.32
6st.	0.47	11.75	0.76	18.45	0.71	9.10	1.09	14.00
7st.	0.58	9.20	1.06	16.82
Top.	1.70	13.19	1.64	12.70	1.98	13.02	3.02	19.88
7L.	2.17	13.30	6.00	36.80
6L.	1.63	9.64	2.09	12.35	1.94	11.15	6.29	36.15
5L.	1.75	8.58	2.24	10.95	2.08	10.40	2.25	11.30
4L.	2.06	10.78	2.49	13.04	1.52	8.54	1.84	10.33
3L.	1.18	7.11	2.34	14.53	1.80	8.77

TABLE 3

SEASONAL CHANGES IN SUCROSE AND STARCH IN NORMAL AND RINGED PLANTS

CANE SEG- MENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)
SUCROSE												
1st ...	0.03	0.133	0.092	0.408	0.13	0.561	0.354	1.525	0.16	0.695	0.378	1.641
2st....	0.11	0.684	0.027	0.167	0.13	0.752	0.000	0.000	0.15	0.834	0.000	0.000
3st....	0.12	0.863	0.454	3.270	0.14	0.972	0.312	2.165	0.11	0.675	0.422	2.530
4st....	0.13	1.192	0.326	2.990	0.07	0.721	0.311	3.200	0.10	0.770	0.883	6.790
5st....	0.13	1.625	0.262	3.270	0.07	0.875	0.291	3.540	0.10	1.020	0.336	3.430
6st...	0.08	1.950	0.213	5.180	0.12	1.540	0.683	8.750
7st....	0.08	1.260	0.149	2.360
Top...	0.32	2.480	0.694	5.380	0.43	2.830	1.826	12.000
7L....	0.39	2.390	1.755	10.875
6L....	1.17	6.930	1.119	6.620	0.60	3.430	2.810	16.125
5L....	1.00	4.910	1.850	9.070	1.75	0.875	0.693	3.465
4L....	0.54	2.830	1.498	7.850	0.81	0.456	0.713	4.000
3L....	0.27	1.623	0.891	5.375	0.59	0.288
STARCH												
1st....	0.15	0.667	0.000	0.000	0.37	1.595	0.112	0.482	1.00	4.350	1.111	4.840
2st....	0.12	0.745	0.025	0.155	0.30	1.735	0.000	0.000	1.28	7.120	0.160	0.890
3st....	0.12	0.854	0.064	0.460	0.29	2.015	0.645	4.482	1.33	8.160	1.870	11.475
4st....	0.13	1.191	0.056	0.511	0.10	1.048	0.608	6.270	1.01	7.770	1.825	14.050
5st...	0.10	1.250	0.062	0.769	0.11	1.376	0.633	7.910	0.86	8.780	1.062	10.850
6st....	0.06	1.462	0.028	0.685	0.48	6.160	1.044	13.400
7st....	0.50	7.940	0.853	13.550
Top...	0.78	6.050	0.166	1.289	1.59	10.460	0.748	4.360
7L....	1.59	9.750	4.360	26.740
6L....	0.28	1.657	0.132	0.778	1.34	7.710	2.355	13.520
5L....	0.27	1.323	0.119	0.582	0.22	1.100	1.300	6.500
4L....	0.25	1.310	0.105	0.549	0.36	2.020	0.989	5.560
3L....	0.26	1.568	0.044	0.265	0.28	1.366

All fractions except sucrose and the reducing sugars in the July series showed a decrease in the basal segment. In all cases the decrease was less in July and August, owing to the duration of the treatment for only three weeks instead of five and a half weeks as in the June series.

TABLE 4
SEASONAL CHANGES IN ACID HYDROLYZABLE SUBSTANCES
IN NORMAL AND RINGED PLANTS

CANE SEG- MENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	Gm.	RESID- UAL DRY	Gm.	CON- TROL RESID- UAL DRY	Gm.	RESID- UAL DRY	Gm.	CON- TROL RESID- UAL DRY	Gm.	RESID- UAL DRY	Gm.	CON- TROL RESID- UAL DRY
		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)
1st....	2.97	13.20	1.214	5.355	5.14	22.15	2.077	8.953	3.32	14.43	2.605	11.325
2st....	2.78	17.28	0.840	5.212	3.80	22.48	2.204	12.740	3.79	21.05	1.634	9.070
3st....	2.39	17.19	3.089	22.230	3.18	22.08	3.343	23.300	2.36	14.47	2.683	16.424
4st....	2.00	18.34	2.152	19.750	1.99	20.52	2.640	27.215	1.80	13.85	2.262	17.390
5st....	1.24	15.50	1.573	19.660	1.68	21.00	2.177	27.250	1.33	13.57	1.425	14.540
6st....	0.78	19.01	0.945	23.020	1.41	18.10	1.206	15.449
7st....	0.78	12.39	0.725	11.500
Top....	1.07	8.29	1.047	8.114	1.86	12.24	1.351	8.888
7L....	2.00	12.25	1.714	10.501
6L....	2.15	12.73	2.093	12.385	2.41	13.86	3.979	22.880
5L....	2.57	12.61	1.603	8.308	1.78	8.90	1.830	9.159
4L....	2.55	13.35	1.995	10.437	1.48	8.32	2.152	12.083
3L....	1.82	10.96	1.894	11.410	1.98	9.67

Sucrose made remarkable gains in the basal segment, in some instances equaling and even surpassing the increase in the segment above the rings. The large increases above the ring, 100 per cent greater than the increases of reducing sugars, indicate very strongly that sucrose serves only as a translocatory form moving downward in the stem. However, increases of the same magnitude below the rings are equally strong evidence that sucrose is the main translocatory form moving upward from the roots into the stem. Thus ringing brings about a bidirectional movement in the canes, which is con-

TABLE 5
DECREASES (−) OR INCREASES (+) AS PERCENTAGE
OF NORMAL IN FIRST THREE SEGMENTS
OF RINGED STEMS

FRACTION	CANE SEGMENT	JUNE 23 (%)	JULY 16 (%)	AUGUST 9 (%)
Total carbohydrates	1st.....	− 58	− 42	− 38
	2st.....	− 70	− 45	− 81
	3st.....	+ 57	+ 25	+ 61
Total acid hydrolyzable	1st.....	− 59	− 59	− 21
	2st.....	− 70	− 43	− 57
	3st.....	+ 29	+ 5	+ 13
Reducing sugars	1st.....	− 53	+ 28	− 25
	2st.....	− 66	− 24	− 59
	3st.....	+102	+ 62	+116
Sucrose	1st.....	+211	+173	+134
	2st.....	− 75	−100	−100
	3st.....	+283	+124	+272
Starch	1st.....	−100	− 70	+ 11
	2st.....	− 79	−100	− 86
	3st.....	− 46	+122	+ 41

TABLE 6
GRADIENTS IN REDUCING SUGARS AND SUCROSE
IN NORMAL AND RINGED CANES

	JUNE 23					AUGUST 9		
	TOTAL (GM.)	GM./CM.	6L TO 6ST (GM.)	5L TO 5ST (GM.)	6L TO 3L (GM.)	TOTAL (GM.)	GM./CM.	7L TO 7ST (GM.)
	1ST TO TOPS					1ST TO TOPS		
Normal								
Reducing sugars ...	+9.40	+0.100	−2.20	+1.1	+2.5	+8.00	+0.07	+4.1
Sucrose.....	+1.62	+0.018	+4.98	+3.3	+4.3	+2.14	+0.02	+1.1
	3ST TO TOPS					3ST TO TOPS		
Ringed								
Reducing sugars....	+1.8	+0.030	−6.1	−3.0	−2.1	+3.0	+0.04	+20.0
Sucrose.....	+2.1	+0.035	+1.4	+5.8	+1.2	+10.5	+0.14	+14.5

TABLE 7
CHANGES IN RESIDUAL DRY WEIGHT IN FIRST FOUR SEGMENTS OF NORMAL AND RINGED CANES
OF JUNE AND AUGUST COLLECTIONS

CASE SEGMENT	MAY 15					JUNE 23					JULY 16					AUGUST 9				
	NORMAL					NORMAL					NORMAL					NORMAL				
	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES * (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)	RESID- UAL DRY WEIGHT (GM.)	TOTAL CAR- BOY- DRATES (GM.)
1st.....	23.5	5.8	22.5	4.0	8.3	1.7	-63.0	-57.5	23.2	6.6	23.0	8.2	19.4	5.1	-15.6	5.1	5.1	-15.6	5.1	-37.8
2st.....	16.0	5.0	16.1	3.7	8.9	1.1	-44.7	-70.4	17.3	5.1	18.0	6.4	13.4	2.3	-25.6	2.3	2.3	-25.6	2.3	-64.0
3st.....	8.2	2.7	13.9	3.3	16.2	5.1	+23.7	+54.5	14.4	4.4	16.3	4.8	20.2	7.7	+23.9	7.7	7.7	+23.9	7.7	+60.4
4st.....	10.9	3.1	11.5	4.3	+5.5	+38.8	9.7	2.8	13.0	3.9	16.8	7.0	+22.6	7.0	7.0	+22.6	7.0	+79.5

* Increase (+) or decrease (-) as percentage of normal.

firmatory of evidence based on virus activity by BENNETT (1). In addition to the accumulation of sucrose above the rings, an accumulation of reducing sugars also occurs, indicating that some of the latter move downward in the bark.

CARBOHYDRATE GRADIENTS

All carbohydrate gradients in the stem are positive; that is, they descend from a high concentration in the top to a low concentration in the base.⁴

The gradients are positive for insoluble as well as for soluble carbohydrates, and therefore do not give evidence of the direction or the form of movement. Girdling may increase or decrease the gradients but they always remain positive.

As was found by MASON and MASKELL (20, 21), sugars move downward with a marked positive gradient (table 6). Unlike their results, however, is the fact that ringing does not cause the gradient to disappear (table 6). It reduces the positive gradient of reducing sugars per stem-centimeter and steepens the gradient of sucrose per stem-centimeter (table 6). Ringing also reverses the positive gradient of reducing sugars from leaves to stem in the fourth and fifth segment of the June series. The sucrose gradient from leaves to stem segment is reduced but remains positive.

The positive gradients of acid hydrolyzable carbohydrates and of starch are stable; that is, they are gradients produced by storage, temporary or permanent, of the two groups of substances. These are static gradients brought about by accumulations within living cells or cells associated with living cells. The proportion of living to "dead" cells is much greater in the tops, upper leaves, and the neighboring subterminal stem segment than in the lower portions. The amount of meristematic activity becomes less in proportion to the amount of secondary xylem, cork, and fibers produced toward the basal segment. An important contributory factor to the positive gradients of the static carbohydrates is the increase from the top to the base of the stem in residual dry weight by which the percentages are determined.

⁴ MASON and MASKELL (20) defined gradients as positive—high concentrations in the upper and low concentration in the lower portions, and negative—the reverse.

The positive gradient of reducing sugars is for the most part a static one of temporary storage sugars accumulated within living cells and associated with metabolism. Sugars may accumulate against a gradient of reducing sugars. MASKELL and MASON (19) demonstrated that movement of sucrose took place with a positive gradient into the cotton boll, in which the concentration of reducing sugar was much higher than in the stem. Reducing sugars thus accumulated as a storage sugar in the boll. Although in the raspberry experiments bark and wood were not analyzed separately, microchemical data show that much of the reducing sugars contributing to the static gradients are stored in the xylem, pith, and rays.

Part of the positive gradient of reducing sugars is undoubtedly translocatory, as is evidenced by the accumulation of these sugars above the ring (table 5), and the consequent decrease of the gradient (table 6). This is also evidence that the bark, and presumably the phloem, is the region in which the translocatory reducing sugars are moving. The data of tables 5 and 6 are also indicative of the downward movement of reducing sugar.

It remains to explain why, despite a 200 per cent increase in sucrose above the rings, the insignificant sucrose gradient actually increases considerably. It would normally decrease, or if nonexistent would become negative, if sucrose were moving downward in the bark. The evidence for downward movement of sucrose shown by one datum (increase above the rings) seems paradoxical in view of the evidence for upward movement by the other datum (increase below the rings). The canes used in these investigations are annual canes, but they are growing from perennial, sugar-gorged roots to which many other canes supply carbohydrates. If translocation of sugar is considered as polar from a source (the leaves) to a "sink" (the roots), downward movement will proceed along a positive longitudinal gradient (20, 21). The situation is different with the raspberry, however, for there is in the young first year canes apparently no well defined polarized movement. DU SABLON (15) explained the April maximum of carbohydrates in the first year canes of the raspberry as brought about by a migration of carbohydrates from the roots. Thus it is apparent that, in the very early stage of development of the vegetative canes, the usual downward movement

of mobile sugars common to most annuals and perennials is reversed; that is, there is established a temporary root-to-shoot gradient, even though the shoot is highly photosynthetic. During the ensuing growth period the early spring root-to-shoot gradient gradually disappears, owing to the increased production of sugars by the rapidly enlarging foliage region of the cane. Reducing sugars are produced in far greater abundance than sucrose, and the leaves-to-root gradient is soon established. Sucrose is formed in small amounts, and seems to be confined to the bark. The production of an abundance of sucrose in the upper regions of the cane is much slower than the production of an abundance of reducing sugars. During the first season, therefore, the downward movement of sucrose seems to be counterbalanced by an upward movement, seemingly in the same channel, and the net result is a static condition with no apparent gradient. As pointed out previously in this paper, the slight gradients of sucrose, as shown in figures 1 to 4 and in table 6, are thought not to be significant because the gradient exhibited is produced by dividing by a higher residual dry weight in the lower segments than in the upper segments. The gradient apparently does not exist. The effect of a ring on this situation is to reverse the influence of the opposing source of sucrose on the other gradient. The result is an accumulation of sucrose in the segment above and below the isolated segment, and a steepening of the positive gradient above the rings. No data were obtained on the gradient below the rings, but on the basis of the preceding explanation a negative gradient would appear. No analyses were made on the roots.

USABILITY OF RESIDUAL POLYSACCHARIDES

Since the introduction by SCHULZE (29) of the term "hemicellulose" to denote a cell wall substance similar in nature to cellulose, ever increasing data point to this group of substances as being of definite physiological significance. DU SABLON (15) demonstrated an accumulation of hemicellulose in the fall and a subsequent utilization in the following spring. SCHELLENBERG (28) showed that hemicellulose is utilized as a reserve food by trees, and CASTORO (5) thought that the hemicellulose which he found in the seeds of *Ruscus aculeatus*

functioned as a reserve food. TOTTINGHAM *et al.* (31) assigned to them the function of serving as reserve carbohydrates in apple trees, and ROSA (27) assigned to them a distinct function in the winter hardening process of plants. NIGHTINGALE (25) presented data which indicated that hemicellulose is utilized by the plant as a source of sugar. MURNEEK (24) discussed the significance of hemicellulose as a reserve carbohydrate in apple spurs. CLEMENTS (6) presented convincing evidence that in the sunflower, soy bean, and potato the hemicelluloses are significantly functional in several roles: (1) they serve as temporary reserves under favorable conditions for carbohydrate synthesis; (2) their accumulation and maintenance add to the drought resistance of the plant; (3) they play a morphic role by contributing to the strength of tissues as cell wall thickenings; and (4) they serve as energy reserves in stem and roots. Many investigations have since been reported in which the role of hemicelluloses in drought resistance has been confirmed. Nevertheless LOOMIS, in a report assembled for the committee on chemical methods of the American Society of Plant Physiologists (12), states with regard to the hemicelluloses, "The physiological significance of these materials has not yet been established." Recently CLEMENTS (9), on the basis of evidence in the soy bean, suggested a mechanism of drought resistance in which the gel-forming properties of the hemicelluloses, which not only line the inner walls of the cells but extend into the protoplasm as well, play an important role.

WINKLER and WILLIAMS (32) have recently submitted data on the hemicellulose content of sections of grape stems which they think refute the contentions of the supporters of hemicellulose as a food reserve. They state, in regard to the work of MURNEEK (23), LEONARD (18), and CLEMENTS (6), that "the observed variations of the hemicellulose content were irregular, and furthermore, are not correlated with the vegetative condition of the plant. Their variations certainly do not point to hemicellulose as a reserve." However, these workers did find an unmistakable correlation of hemicellulose fluctuations with the vegetative condition of the plant (6, 9, 10). WINKLER and WILLIAMS found no significant variations in hemicellulose content of grape stems. They showed no data for the other carbohydrate fractions.

In all the work just mentioned the hemicelluloses were determined following acid hydrolysis of not more than six hours' duration. In this investigation the term acid hydrolyzable carbohydrates has been substituted for hemicellulose; furthermore, hydrolysis was carried out for fifteen hours. The quantities of substances extracted in this manner showed large variations in different parts of the plant, attesting their possible role as temporary food reserves.

The hemicelluloses have thus been established as playing a significant physiological role in metabolism. As such they are considered the most stable of the important carbohydrates which are actively associated with metabolism. Their function in metabolism is variable among plants, and depends to a great extent upon the kinds and amounts of other more readily utilizable carbohydrates. Their role may be a minor one in the normal life of some plants under good conditions for growth (32), but a change in conditions of growth will usually find its effect in the hemicelluloses. Soy beans react more favorably to conditions of drought than do potatoes and sunflowers, owing primarily to a greater abundance of hemicelluloses (9, 10).

Because they are a heterogeneous group of polysaccharides and their derivatives, it is extremely difficult accurately to define their chemical composition. It is likely that this group includes much of the "intussusception" material of the cell walls. The less dehydrated material is hydrolyzed first and in greatest amount. This is the material which is most available as hemicellulose reserve, and it is commonly obtained within a three hour period of hydrolysis. Hydrolysis for a fifteen hour period should yield most of the hemicellulose available for utilization in metabolism.

The total carbohydrates, which in this work included the substances hydrolyzable in 2.5 per cent acid in fifteen hours, were subtracted from the dry weight of the sample, and the residual dry weight was obtained as suggested by MASON and MASKELL (20). The residual dry weight consists of minerals (about 3-4 per cent of the dry weight, 7), fats, total nitrogen, and the stable cell wall material, including cellulose, which is not hydrolyzed in fifteen hours. The total organic nitrogen makes up 1-3 per cent of the residual dry weight, and fluctuates within this range. These substances—fats, minerals, and total organic nitrogen—do not at most account for

more than 5 per cent of the fluctuations in residual dry weight. In the ringed canes the latter deviates as much as 63 per cent of the normal (table 7). The great fluctuations in residual dry weight are therefore owing to fluctuations in residual polysaccharides.

The distributions, in grams per sample, of the residual substances in the first four segments of normal and ringed canes of the June and August series are given in table 7. Included in the table for comparison with the distributions of the June and August series are the figures for the May series. The deviations from the normal of the residual substances in the ringed canes are given in percentages. In the normal canes the residual substances, like the carbohydrates, are distributed in decreasing weights from the base toward the top. The weight gradient in grams is therefore opposite (negative) to the concentration gradient. In the first two segments the residual dry weight of the normal canes remained fairly constant throughout the season.

In the ringed canes the fluctuations of residual substances are in the same direction as those of the total carbohydrates. Where there is a loss of carbohydrates (see May and July figures, table 7), there is a loss of residual substances; and where there is an accumulation of carbohydrates there is a large increase in residual substances. An inversion of the residual polysaccharides takes place as the amount of carbohydrates is depleted by respiration. Above the rings the accumulation of total carbohydrates is faster than the condensation to the residual polysaccharides, owing to the influx of soluble forms from the foliage region. The evident responses of the residual polysaccharides to fluctuations in the total carbohydrates are definite indications of the equilibrium between these two groups. If a sufficiently large shift is produced by depletion or accumulation of the soluble forms, this shift will ultimately produce its effect in the residual polysaccharides. There are no data available which indicate how quickly the adjustment occurs, but the results presented here leave little doubt that it does take place.

COORDINATION OF RESULTS

In the normal distribution of the soluble carbohydrates the reducing sugars are far more abundant than sucrose. This abundance is

superseded only by the acid hydrolyzable substances whose activity seems to be closely associated with the reducing sugars. Their persistent abundance, coupled with the positive gradient of reducing sugars, is associated with the gradient of living cells. This leaves no doubt that the reducing sugars are the major immediate source of metabolic energy, as well as a temporary storage form. Removal of a ring of bark causes an accumulation of the reducing sugars above it, and in so doing decreases the gradient in the stem. These results indicate that a portion of the reducing sugars is functional in translocation in the bark. The positive gradient of reducing sugars in the normal canes is the sum of two components: (1) a static positive gradient of temporary storage sugars associated with active protoplasm; and (2) a dynamic positive gradient of reducing sugars which are translocatory.

The concentration of sucrose is very low at all times of the season. However, it is by far the most responsive to ringing of all forms, and this sensitivity is evidence of its role in the metabolism of the plant. Sucrose apparently is not produced in the raspberry by a fructose-glucose concentration maximum. Its gradient, if it has one, is so slight that it is well within the error produced by variations of the residual dry weight upon which the concentrations are expressed. It is thought that no gradient exists. Ringing causes a 200 per cent (of the normal) accumulation above and below the rings, and a 100 per cent decrease between the rings. The slight positive gradient which might exist is made more positive; if the gradient does not normally exist, a positive one appears. These data are interpreted to mean that:

1. Sucrose serves essentially as a translocatory form.
2. It moves in the bark, probably in the phloem.
3. There is an upward movement of sucrose from the perennial roots into the young shoot. It is probable that some of this sucrose is supplied by normal canes growing from the same roots.
4. As the developing cane enlarges and produces an increasingly greater photosynthetic area, sucrose is formed in the upper region of the cane and tends to move downward with its own gradient.
5. During the growth of the first year canes there is a period of adjustment of the two sources of sucrose. The gradient of one is

compensated by that of the other, and no gradient appears. It is likely that in the second year a definite positive gradient of sucrose appears, owing to the preponderance of the sugars produced by a large photosynthetic cane.

6. Ringing removes the influence of one source of sucrose upon the other, and an accumulation occurs above and below the isolated segment. A gradient, which before was compensated by one issuing from an opposing source, is made to appear.

It is significant that sucrose entirely disappears from the segment between the rings, whereas the reducing sugars are always present, although low in quantity. Unlike the reducing sugars, sucrose does not appear upon hydrolysis of the "hemicelluloses"; therefore sucrose is not a product of hydrolysis. Perhaps it is formed, in a manner similar to that found in the cotton plant by PHILLIS and MASON (26), within the sheath cells or companion cells of the phloem, and appears essentially in the phloem. In the raspberry it seems to have no function other than one of translocation.

Starch plays an insignificant role in the metabolism of the raspberry. BRIERLEY and LANDON (4) were unable to demonstrate microchemically the presence of starch, even in the fruiting canes. The acid hydrolyzable substances were shown to be intimately associated with the reducing sugars, being in equilibrium with them and fluctuating in the same direction. When the fluctuations in reducing sugars are sufficiently great, not only do the acid hydrolyzable substances respond, but also the residual polysaccharides. The latter are thought to include the remaining portion of the "intussusception" material of the cell wall which is not taken out in the acid hydrolyzable fraction.

Thus it appears that all carbohydrates, with the probable exception of the primary cellulose wall, are actively or potentially functional in the metabolism of the plant. The "exceedingly complex dynamic equilibrium" of which SPOEHR (30) writes extends beyond the hemicelluloses to include the cell wall polysaccharides and their derivatives. The activity of this group in carbohydrate metabolism depends upon: the kind of plant; the amounts of other carbohydrates present; the nature and stability of the polysaccharides and

derivatives making up the hemicelluloses; the residual substances; and, what is still more important, the magnitude of the shift in the "dynamic equilibrium."

Summary

1. The seasonal fluctuations in reducing sugars, sucrose, starch, acid hydrolyzable carbohydrates, total carbohydrates, and the residual polysaccharides are recorded for the raspberry plant.

2. Double rings, one 15 cm. and another 30 cm. above the ground, isolating a 15 cm. segment of stem, were used to check movements of sugars.

3. In normal plants the concentration of total carbohydrates is fairly high in May, drops during June, the period of most active growth, and then increases to a maximum in August. The acid hydrolyzable carbohydrates are the largest fraction of the total carbohydrates. The concentration of reducing sugars is highest in May, drops rapidly in June, and increases slightly in July and August. A low concentration of sucrose is present at all times. Starch is never abundant, and apparently is not an important carbohydrate reserve.

4. In ringed plants the total carbohydrates accumulate above the rings, and decrease below and between them. Sucrose made the largest increase above the ring. Reducing sugars accumulated above the rings, but decreased between and below them. Apparently sucrose tends to move upward as well as downward in the bark. It does not appear to be a product of hydrolysis of hemicellulose in the isolated segment as do reducing sugars. A portion of the reducing sugars is translocatory, moving downward in the bark.

5. All carbohydrate gradients are positive. Sugars move downward with a marked positive gradient. The positive gradients of the acid hydrolyzable substances and of starch are static. These gradients are produced by temporary storage brought about by accumulations within living cells and cells associated with living cells. Ringing decreases the positive gradients of reducing sugars, indicating that some of them are mobile in the bark along a dynamic positive gradient. Much of the reducing sugars may be a temporary storage

form and not functional in translocation. Therefore their positive gradient is the result of a positive dynamic gradient of mobile sugars and a positive static gradient of temporary storage sugars.

6. The insignificant and probably non-existent gradient of sucrose is increased rather than decreased, as would be expected if this sugar were moving only downward.

7. The residual substances consist of those polysaccharides, small quantities of fats, minerals, and organic nitrogen which remain after the total carbohydrates, including acid hydrolyzable substances, are subtracted from the dry weight. The residual polysaccharides are thought to include the "intussusception" material (polysaccharides of high molecular weight and their derivatives) of the cell wall. The residual polysaccharides fluctuate as the total carbohydrates (particularly reducing sugars and the "hemicelluloses") fluctuate, and in the same direction.

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EXPERIMENTS ON THE TRANSPORT OF AUXIN¹

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(WITH TEN FIGURES)

Introduction

The purpose of the work described in this paper was to measure quantitatively the transport of auxin, using a new experimental technique. The original method of measuring the rate of auxin transport required the determination of the amount transported through the tissue to an agar block during a series of time intervals, thus determining the time at which the first auxin arrived (6). Since with the photokymograph (4) the beginning of the curvature can be accurately determined, and since this initial curvature occurs a known time interval after application of auxin to the recording plant, it is possible to determine for each coleoptile when auxin reaches its cut surface. Thus by placing a piece of tissue one-sidedly on a recording plant, and applying auxin to the other end of this tissue, the rate of auxin movement through it can be determined.

Method

Preliminary experiments were all unsuccessful for one of two reasons. When coleoptile sections were interposed between the recording coleoptile and the agar block containing auxin, either the curvatures started simultaneously with the controls as if no section were present, or no curvatures appeared at all. Finally, however, the technical difficulties were overcome, and transport velocities of the same order of magnitude as VAN DER WEIJ (6) described were obtained. To this end the following improvements in technique had to be made, for the rest adhering to the procedure previously outlined (4). Figure 1 gives a sketch of the new set-up.

The grass peduncle inserted in place of the primary leaf did not give a satisfactory support of the applied tissue plus agar block, and

¹ This work was carried out with the aid of the Works Progress Administration, Official Project no. 165-03-6999, Work Project no. 6330-6989.

the latter often stuck to it. The method by which the leaf is partly pulled out and a fine metal wire (*D*) is inserted gave good results, provided the agar block (*A*) and the top of the transport tissue (*B*) did not touch the wire. It was also found that copper wire gave irregular results when not properly inserted

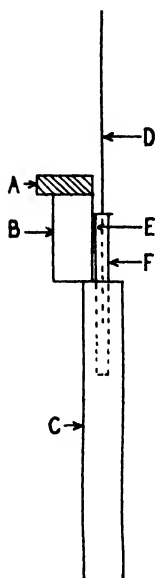


FIG. 1.—Schematic picture of test plant *C*, with silver wire *D* inserted in the partly pulled out primary leaf *F*. On one side of its cut surface the transport section *B* is stuck by means of petroleum jelly *E* against the primary leaf *F*; on top is an agar block *A*, containing the substance to be transported.

and touching the coleoptile walls; nichrome wire and especially silver wire nr. 36 (diameter 0.12 mm.) were very satisfactory.

The primary leaf (*F*), after pulling loose, was cut off so that the transport tissue would protrude about 2 mm. above it. A thin film of petroleum jelly (*E*) was applied to the upper part of the protruding part of the primary leaf, care being taken not to come in contact with the cut surface of the coleoptile as it would interfere with the transport of auxin. The transport tissue (*B*) was then placed on the cut surface of the recording coleoptile (*C*) and held in place by the petroleum jelly (*E*) against the primary leaf (*F*). The contact between the tissues is very essential and was therefore inspected with a magnifying glass. The agar blocks (*A*) containing the growth promoting substances were then placed on top of the transport tissue, so that they would not touch the wire and the contact between transport tissue and

recording plant would not be broken. In all experiments, blocks of $2 \times 2 \times 1$ mm. 2 per cent agar were used, which were washed in the solutions for 1–2 hours.

To remove the auxin still contained in the tissues through which transport had to be measured, and for which mostly *Avena* coleoptile sections were used, these were placed with their basal cut surface on wet filter paper for 3 hours. After that they did not induce curvatures by themselves when no auxin was applied.

The most important departure from the standard conditions as described by SCHNEIDER and WENT (4), however, was by decreasing the relative humidity of the air to 75–77 per cent during the test. This unfortunately causes premature drying of the agar blocks, but for the first 3 hours the decrease in volume is not considerable. This lowering in humidity is essential to prevent leakage of auxin along the surface of the transport tissue. That leakage actually occurred at high humidities was found by placing inverted coleoptile sections on the recording plants and applying high concentrations of indoleacetic acid in agar on them. When this was done at 85–90 per cent humidity strong curvatures resulted; at 76 per cent humidity there was no curvature at all, and therefore no transport through the inverted sections. In normal sections, of course, considerable transport took place. At 88 per cent humidity lanolin paste containing 0.1 per cent indoleacetic acid gave no inverse auxin transport through 6 mm. coleoptile sections, since no auxin would leach from the paste. Further details of this polar transport are given later in this paper.

For the transport test, the recording plants (as well as the sections) were even more carefully selected than in the standard *Avena* test. Among other factors, this selection was for thick coleoptile walls, which gave a better contact surface. Plants 30 mm. long were found best.

In general the reaction of the recording plants was delayed, owing to the extra time required for the auxin to reach the plant through the transport tissue. Since in the *Avena* test plants, sensitized through a double decapitation, the “regeneration” opposes the auxin curvature after 80 minutes, the standard double decapitation method (10) was not suitable for this transport test. Therefore the coleoptiles were decapitated only once, and the transport tissue with auxin was applied immediately, so that the curvature could occur before the regeneration (4, p. 477). To make the control plants comparable, the agar blocks without transport tissue were applied 40 minutes (mean transport time) after decapitation.

Evaluation of results

All these transport experiments have been repeated several times; the reaction of a total of more than 2000 plants has been recorded in

all. Only about one-sixth of this material is here presented, since all results were closely similar. Some of this additional material, however, is incorporated in tables 2 and 3.

Before the experiments are discussed in detail, certain difficulties in the interpretation of the data should be pointed out.

On account of the variability of the material, some method of averaging is essential. As previously described (4), the position of

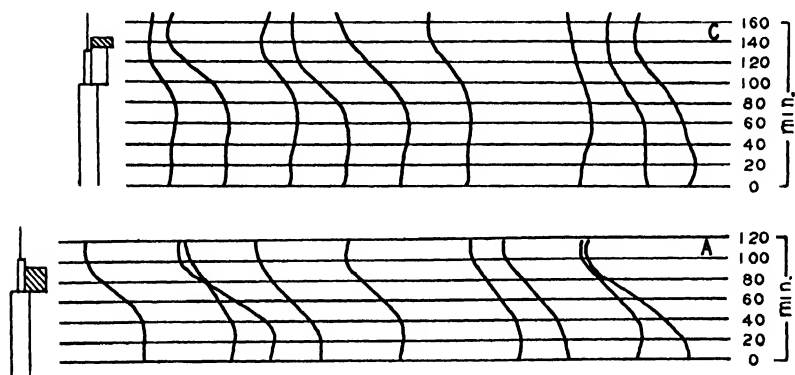


FIG. 2.- Retracing of part of record of experiment represented in fig. 3. Parallel lines drawn at 20 min. intervals, extending between them. Where they are perpendicular to the time lines the plants remain straight; slope toward left means negative (growth) curvature. Curve A: controls, curvatures start 20-40 min. after first recording. Time elapsed between putting on of agar block (containing 0.1 mg./l. indoleacetic acid) and first recording, 4 minutes. Curve C: 6.3 mm. coleoptile section inserted between agar block (containing 4 mg./l. indoleacetic acid) and test plant. Curvatures start 60-80 min. after first recording, which occurred 8 minutes after putting on the agar blocks.

the tip of the test plant is recorded once every 4 minutes. As long as the plant does not curve, this position remains the same, and a straight line is recorded (fig. 2). Although in general the onset of the curvature is very sudden, so that it should not be difficult to determine in which 4 minute period the curvature starts, yet unfortunately the nutations which are always present to some extent make it difficult to measure this point with the desired accuracy. When the rate of curvature is small, it is also difficult to tell when the curvature started. By taking the mean of the reactions of eight to ten plants, these nutations are ruled out and the onset of the curva-

ture can be determined with greater accuracy from the mean curve, as shown in figures 3-10.

But a new difficulty arises: in these mean curves the beginning of the curvature will not be the mean of the onset of the individual plants, but will depend upon the moment that the first one starts to bend. Therefore there must be a systematic difference between the two types of calculation (table 1). Figure 2 is a retracing of the record of the experiment represented in figure 3, curves *A* and *C*.

TABLE 1

TIME BETWEEN APPLICATION OF AUXIN AND (1) ONSET OF CURVATURE OR (2) INTERSECTION POINT, MEASURED FOR INDIVIDUAL TEST PLANTS AND CALCULATED FROM AVERAGE CURVES OF FIGURE 3. FOR A DESCRIPTION OF INDIVIDUAL TREATMENTS (*A*, *B*, *C*, *D*), SEE FIGURE 3

CURVE	TYPE OF READING	INDIVIDUAL TIMES IN MINUTES (MEASURED FROM FIGURE 2)	MEAN	CALCULATED FROM FIG. 3	MEAN OF THREE SHORTEST TIMES
A.....	Onset	20, 28, 36, 32, 32, 32, 28, 28, 44 ..	31	26	28
	Intersection	34, 42, 44, 38, 46, 38, 42, 40, 52 ..	42	41	37
B.....	Onset	38, 42, 30, 46, 34, 34, 34, 42, 46 ..	38	32	33
	Intersection	36, .., 44, 46, 48, 32, 38, 58, 46 ..	43	40	35
C.....	Onset	76, 68, 72, 60, 72, 80, 76, 80	73	67	67
	Intersection	80, 86, 96, 84, 84, 94, 96, 94	89	87	83
D.....	Onset	60, 64, 56, 72, 64, 60, 56, 80, 64, 64	64	55	57
	Intersection	62, 76, 72, 74, 76, 78, 74, 80, 86 ..	75	73	69

The individual reactions are all similar, and in no single case can there be doubt that the transport section greatly delayed the onset of the curvature. For the rest the type of reaction is the same. After a short start phase, the rate of curvature becomes constant (eumotonic phase), remains so for 30 to 60 minutes, and then decreases. The eumotonic phase can be used for an unambiguous determination of the beginning of the curvature. If a line is drawn extending this eumotonic phase it will cross the line which extends the original position of the plant, and the intersection between the two lines is easy to determine and is a much more definite point than the onset of the curvature (points *A* and *D*, fig. 3). This point also depends

on the moment of arrival of the auxin at the cut surface. But the mean of the individual intersections does not significantly differ from the intersection derived from the average curve (table 1). Besides, this point of intersection is more or less independent of the auxin concentration. This is not true of the time of onset of curvature, which can be detected the sooner the greater the rate of curva-

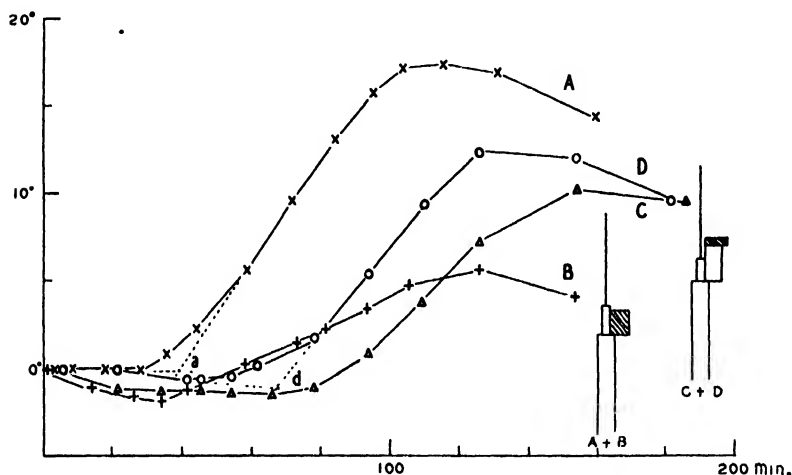


FIG. 3. Abscissa: time in minutes after application of auxin. Ordinate: movement of tip of test plants in degrees. Curves A: 0.1 mg./l. indoleacetic, applied directly; B: 0.05 mg./l. indoleacetic acid, applied directly; C: 4 mg./l. indoleacetic acid, applied on intervening 6.3 mm. upright coleoptile section; D: as C, but concentration 2 mg./l. indoleacetic acid.

ture (= rate of auxin arrival; table 1). These considerations sufficiently justify the use of this intersection point throughout this paper.

BASIC EXPERIMENTS

Figure 3 shows the course of the curvature of the test plants when agar blocks containing auxin were placed on directly, or with an intervening coleoptile section of 6 mm. Each curve is calculated as the mean of eight to ten test plants. Figure 2 and table 1 give data on the individual reactions in this experiment. A few conclusions, substantiated by other experiments which will not be described here, can be drawn from figure 3. In the first place it is seen that the slope of the curve (that is, the rate of curvature) depends upon the con-

centration of the auxin in the agar block (curves *A* and *B*; see also curves *A* and *B*, fig. 6). This is the same as described before (4, p. 488). There is no difference in the rate of curvature of the test plants carrying the transport sections (curves *C* and *D*), indicating that they represent "maximum angles"; that is, the auxin concentration reaching the test plants was so high that they curved at a

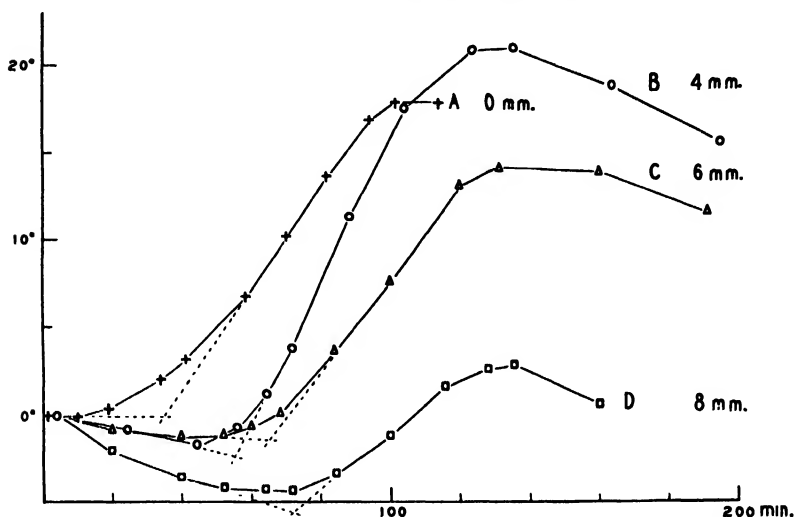


FIG. 4.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of tip of test plants, in degrees. Curves *A*: agar directly applied to test plant; *B*: applied on 4.2 mm. transport section; *C*: on 6.3 mm.; *D*: on 8.4 mm.

maximal rate. The type of the curves *A*, *C*, and *D* is similar, with the exception of the moment when the plants start to curve. The transport time of auxin through 6.3 mm. sections was 39.5 minutes in this experiment, corresponding to 9.6 mm./hour.

The experiment of figure 4 compares well with that of figure 3. Each curve was calculated as the mean of eight to ten test plants. The transport time through 4.2 mm. sections (curve *B*) was $55-35 = 20$ minutes, for 6.3 mm. sections (*C*) $65-35 = 30$ minutes, and for 8.4 mm. sections (*D*) $77-35 = 42$ minutes. Thus the conclusion of VAN DER WEIJ (6) is confirmed, that the velocity of transport is independent of the length of the coleoptile section, in this experi-

ment being 12.4 mm./hour. The amount transported, however, decreases with the longer sections.

Figure 5 shows the auxin transport through sections cut from different levels of the coleoptile. In agreement with the experiments of VAN DER WEIJ (6) with transport of an auxin concentrate from urine, it was found that the sections cut nearest the tip (*B*) transported somewhat more auxin than middle sections (*C*), but basal

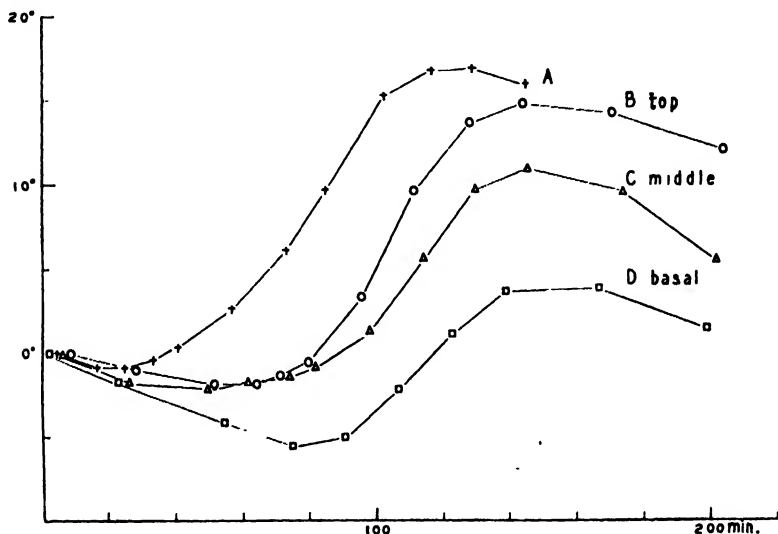


FIG. 5.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of test plants in degrees. Curves *A*: control, agar directly applied to test plant; *B*, *C*, *D*: with interposition of 6.3 mm. coleoptile sections (*B*, cut 3–9 mm. from tip, *C* 9–15 mm., and *D* 16–22 mm.).

sections gave distinctly less transport (*D*). The velocity differed very little in the three regions of the coleoptile. Since VAN DER WEIJ did not find differences in transport capacity at various heights in the coleoptile, when *Zea* tip auxin was used, it would indicate that his auxin concentrate resembled the indoleacetic acid which we used in our experiments rather than the auxin *a* or *b* from *Zea* coleoptile tips. These experiments indicate that never more than the upper 9 mm. of the decapitated coleoptile should be used for transport sections.

Since only a small fraction of the cut surface of the transport sec-

tion is in contact with the test plant, it was of importance to re-investigate the auxin transport through the tissues containing the vascular bundles, and through the parenchyma. On the cross section the coleoptile is elliptical, and the two vascular bundles extend along the narrow side. In two experiments, one of which is summarized in figure 6, the transport sections were attached to the test

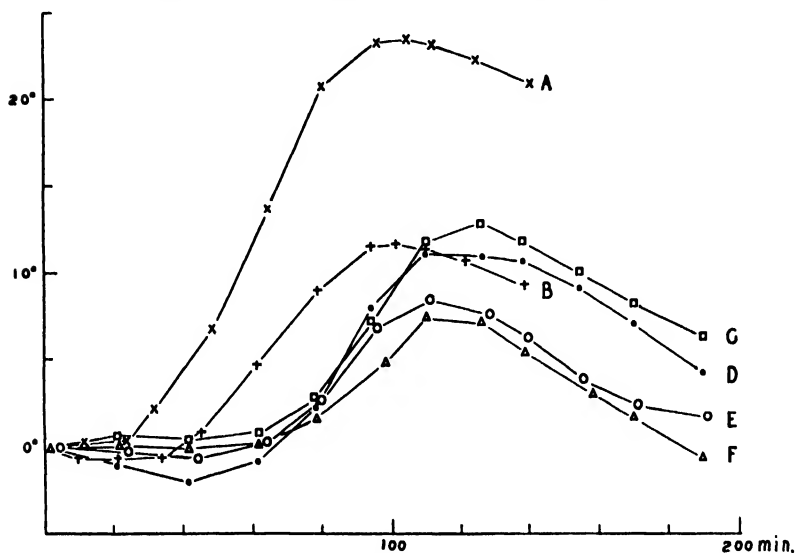


FIG. 6.—Abscissa: time in minutes after application of indoleacetic acid 0.25 mg./l. (curves A, C, D) and 0.1 mg./l. (curves B, E, F). Ordinate: curvature of test plants in degrees. Curves A and B: controls, agar directly applied to plant. Curves C-F: 4.2 mm. coleoptile section between agar and test plant, in C and E: vascular bundle of transport section in contact with test plant, D and F, parenchyma only.

plants either with their broad (D and F) or with their narrow (C and E) sides. Both experiments gave identical results, showing that the auxin transport is practically independent of the position of the transport section; in other words, that the presence of the vascular bundle does not materially increase the auxin transport. This is in agreement with the results of VAN DER WEIJ (6) and WENT and THIMANN (10), but differs from the conclusions of LAIBACH and KORNMAN (3). Although no difference was found in regard to the position of the transport sections, still in all experiments care was taken to place them with their narrow side against the test plant.

POLARITY OF TRANSPORT

There were many reasons for investigating the polarity of auxin transport with the new technique. In the most accurate transport experiments (6, 7) the mean transport of twelve coleoptile sections was always determined, so that occasional leakage through one or more sections could not be detected. Besides, as mentioned under Method, the previous experiments on auxin transport in the *Avena* coleoptile were all carried out at a high humidity, which al-

TABLE 2

CONCENTRATIONS OF INDOLEACETIC ACID APPLIED DISSOLVED IN AGAR OR IN LANOLIN, TESTED WITH RESPECT TO THEIR ABILITY TO MOVE FROM BASE TO APEX THROUGH AVENA COLEOPTILE SECTIONS OF DIFFERING LENGTH

LENGTH OF COLEOPTILE SECTION (MM.)	NO INVERSE TRANSPORT WITH CONCENTRATIONS		INVERSE TRANSPORT WITH CONCENTRATIONS	
	MG./L. SOLUTION IN AGAR	MG./GM. LANOLIN	MG./L. SOLUTION	MG./GM. LANOLIN
2 1.	10
3 1.	50, 200	2, 1	1000	5?, 10
4 2.	100	10	1000
6 3.	2, 4, 10, 50, 200, 1000	1
10 5.	4

lows a certain amount of auxin leakage along the surface of the coleoptile sections. This leakage must have been considerable, especially in JOST and REISS' (2) experiments, since their coleoptiles were made to adhere to the wall of the glass vessel by capillary action of the solutions containing auxin. Thus it is not surprising that they failed to obtain clear evidence for polar auxin transport. Also it would now seem that part of the inverse transport reported by WENT (9) might have been due to leakage along the surface of the coleoptiles, since the transport experiments were carried out in closed petri dishes in a high humidity. However, the falling off of the transport beyond concentrations of 1 mg. indoleacetic acid/cc. is proof that not all of the inverse transport was due to leakage.

At humidities above 80 per cent, applying auxin in agar, no transport tests showed either polarity of the auxin movement

through the tissues or an appreciable time lag of the auxin curvature when a coleoptile section even in normal position was interposed between agar block and test plant. However, when not auxin agar but auxin paste (1 mg. indoleacetic acid per gram lanolin) was used, even

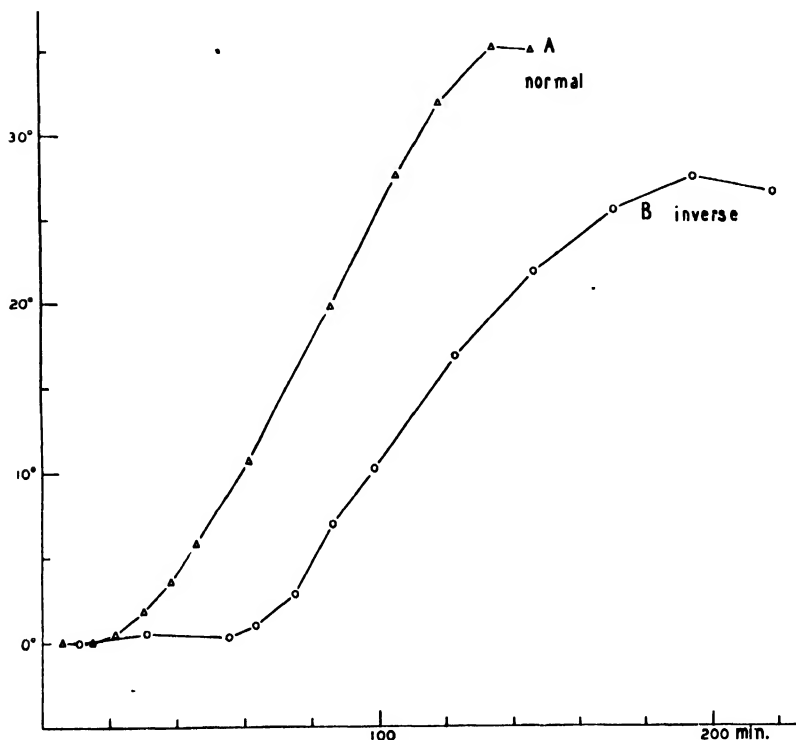


FIG. 7.—Abscissa: time in minutes after application of indoleacetic acid 0.5 mg./l. (curve A) and 1000 mg./l. (curve B). Ordinate: curvature of test plants in degrees. In curve A, a 3.1 mm. coleoptile section was placed apex up between test plant and agar; in curve B this section was placed apex downward (inverse transport).

at a humidity of 85–90 per cent no trace of leakage or non-polar transport was observed.

Since auxin concentrations are physiologically better defined in agar than in lanolin, most transport experiments were carried out with auxin-agar at humidities of 70–80 per cent, with occasional checks with auxin-lanolin pastes. Table 2 summarizes the experiments on inverse auxin transport (each determination is the mean of

ten to twenty test plants). In all cases control determinations were made with coleoptile sections in the normal position, and in every case good transport of a 0.5, 1, or 2 mg./l. solution of indoleacetic acid was obtained, indicating that the lack of transport through the inverted sections was not due to unfavorable experimental conditions. The data in table 2 leave no doubt that the longer the sec-

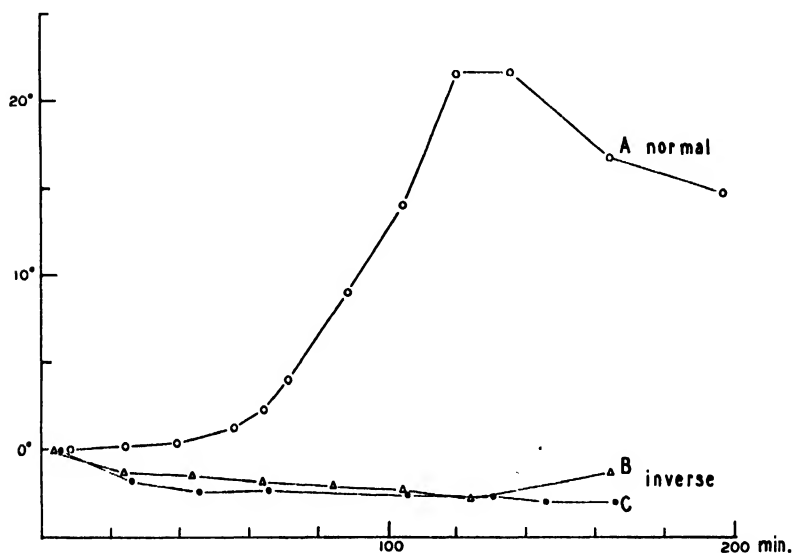


FIG. 8.—As fig. 7, but length of coleoptile section 6.3 mm. Curves A: indoleacetic acid 1 mg./l., section apex upward; B: indoleacetic acid 200 mg./l., section apex downward; C: indoleacetic acid 1000 mg./l., section apex downward.

tions the more strictly polar is the transport. Whereas the 3.1 and 4.2 mm. sections still permit the passage of 1000 mg./l. indoleacetic acid solutions, the 6.3 mm. sections do not do so any more. Figures 7 and 8 give the results of two actual experiments. In each case it was observed that the velocity of inverse transport was considerably less than of normal transport (4–6 mm./hour against about 10 mm./hour), whereas the capacity was greatly less, especially when the enormously increased concentration gradient is considered. Thus the inverse transport differs from the normal transport in more than one way.

TRANSPORT OF DIFFERENT COMPOUNDS

With the described test it is possible to measure the velocity and capacity of transport through coleoptile tissues of all substances active in the *Avena* test. This has been done with indole(3)acetic acid (figs. 3-8), indole(3)butyric acid, anthraceneacetic acid (fig. 9), naphthaleneacetic acid (fig. 10), indole(3)propionic acid, and

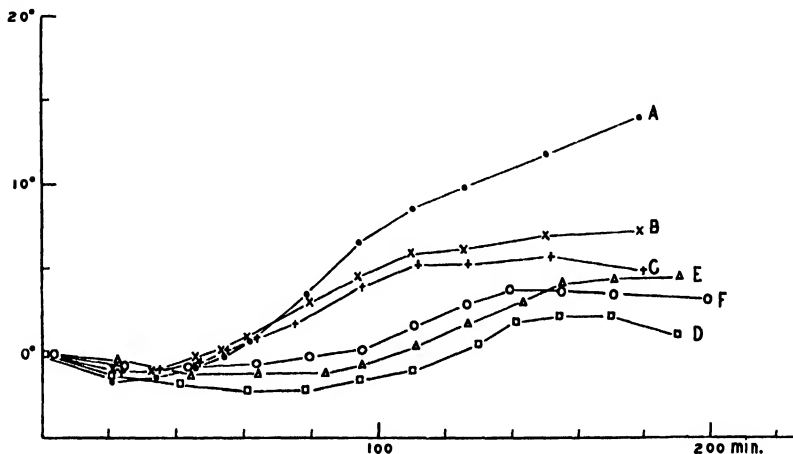


FIG. 9.—Abscissa: time in minutes after application of anthraceneacetic acid. Curves A-C, no sections; D-F, 4.2 mm. sections between agar and test plant. A: concentration 4 mg./l.; B: 2 mg./l.; C: 1 mg./l.; D: 100 mg./l.; E: 50 mg./l.; F: 20 mg./l. Ordinate: curvature of test plants in degrees.

cis-cinnamic acid. The last two did not show any transport at all under the conditions described, and will not be considered further. This was not due to a lack of response of the test plants, since these substances gave curvatures in the standard *Avena* test. In the first four compounds mentioned, however, good values for transport velocity were obtained, and these are given in table 3. The figures in this table are means of three to ten separate determinations with ten plants each.

The figures of line A are calculated from the controls in each experiment, and indicate the time required for the beginning of the curvatures after application of the agar containing the active substance. These figures give an indication of the velocity of the growth reaction in which these compounds must take part.

The second line (*B*) is the reciprocal value of the transport velocity of these compounds. It will be seen that, although there is great variation in the individual determinations, still there is no doubt that the transport of naphthaleneacetic acid is considerably slower than that of indoleacetic acid. This is not due to differences in the applied concentrations, since figures 3, 6, 9, and 10 show that there is no systematic difference between the transport velocities, or the moments of incipient curvature in the controls, when different concentrations are tested.

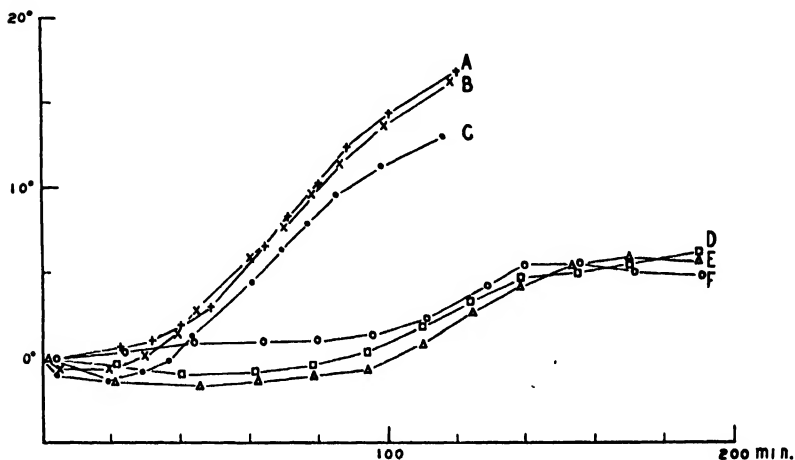


FIG. 10.—Abscissa: time in minutes after application of naphthaleneacetic acid. Curves *A-C*: agar applied directly; *D-F*: 4.2 mm. coleoptile sections between agar and test plants. *A*: concentration 4 mg./l.; *B*: 2 mg./l.; *C*: 1 mg./l.; *D*: 50 mg./l.; *E*: 20 mg./l.; *F*: 10 mg./l. Ordinate: curvature of test plants in degrees.

Line *D* is a summary of results obtained with the standard *Avena* test, and indicates the length of the curved zone 90 minutes after one-sided application of the various compounds. Also in these experiments no differences in length of curvature were observed if different concentrations were applied, which also indicates that transport velocity is independent of concentration. VAN DER WEIJ (6) has come to the same conclusion.

It is interesting to calculate with the aid of lines *A* and *B* how long the curved zone in an *Avena* test would be. Since the transport experiments were carried out with *Avena* plants which were decapitated only once, which retards the onset of the curvature 10 to 20

minutes (4, p. 477), and since the apparent transport is slowed down owing to two extra cut surfaces, the calculations were made for a reaction time of 118 minutes. The calculated values obtained in this way (line C) are in good agreement with the actually measured length of the curved line in the standard *Avena* test (line D).

There is another point which is of interest. Applied indoleacetic acid may give a maximal curvature, even when it reaches the test plant through a transport section, indicating that the amount

TABLE 3

RATE OF GROWTH REACTION (A); TRANSPORT RATE (B); LENGTH OF CURVED ZONE AS CALCULATED FROM A AND B (C); AS DETERMINED IN STANDARD AVENA TEST (D); AND ACTIVITY OF FOUR GROWTH PROMOTING SUBSTANCES IN AVENA COLEOPTILES

	INDOLE(3)ACETIC ACID	INDOLE(3)BUTY- RIC ACID	ANTHRACENE- ACETIC ACID	NAPHTHALENE- ACETIC ACID
A. Time required for beginning of curvature in controls...	37 min.	53 min.	47 min.	34 min.
B. Time required for transport through 4.2 mm. sections...	28 ± 1.9 min.	38 ± 5.3 min.	47 ± 1.8 min.	66 ± 3.8 min.
C. Calculated length of curved zone after 118 minutes...	12.1 mm.	7.2 mm.	6.3 mm.	5.4 mm.
D. Length of curved zone in standard <i>Avena</i> test.....	11.8 mm.	7.8 mm.	5.5 mm.	6.0 mm.
Concentration giving 5° curvature in standard <i>Avena</i> test	0.05 mg./l.	1 mg./l.	1 mg./l.	2.5 mg./l.

transported is considerable. From figure 6 it would appear that almost as much indoleacetic acid reaches the test plant through the interposed tissue as from the agar block directly. This is not true for indolebutyric acid, naphthaleneacetic acid, and anthraceneacetic acid. At concentrations of 10-20 mg./l. practically as much active material reaches the test plant through the transport section as at a five times higher concentration. But the controls indicate that the plants are able to give a much larger reaction. Thus it is clear that, for those substances, the amount transported (or the transport capacity) is limited.

TRANSPORT THROUGH DIFFERENT TISSUES

The method described in this paper can be used to measure the auxin transport through other plant tissues also. Only they should be small enough so that they can be fitted on an *Avena* coleoptile. In a few cases very rapid transport was obtained through *Nitella* internodal cells.

Small parallelepipeds were cut from potato tubers in longitudinal and radial direction, but in no case was any transport found, neither of 10 nor 2 mg. indoleacetic acid per gram lanolin.

In corn coleoptiles good transport was found, but only when high concentrations were applied (10 mg. indoleacetic acid per gram lanolin). In one experiment the transport of low auxin concentrations through 4.2 mm. *Avena* coleoptile sections gave maximum angles, with 59 and 62 minute intersection points in two sets of ten plants each, and no inverse transport occurred. In the 4.2 mm. corn coleoptiles only small curvatures resulted with high auxin concentrations, intersection point 87 minutes (eight plants). Also inverse transport of this same concentration took place, intersection point 102 minutes (ten plants).

Through 4.2 mm. papaya leaf stalks and midribs only very little was transported (120, 128, and 132 minute intersection points), but good results were obtained with 3.1 mm. sections of the same material.

Through young hypocotyls of tobacco plants no transport was observed with 1 mg. indoleacetic acid per gram lanolin, but the same concentration very clearly moved only from apex to base through *Tropaeolum* leaf stalks and flower stalks.

Thus this technique allows the determination of auxin transport through many different types of tissues. It is interesting to note that in general the velocity and capacity of transport were much less than in *Avena* coleoptiles. This work will be continued.

Discussion

It is not necessary to repeat the discussion on polarity of the auxin transport, as given by WENT and THIMANN (10). The present paper gives additional and rigid proof of the almost absolute polarity of the *Avena* coleoptile tissue for the transport of indoleacetic acid. It

also indicates why some investigators, especially JOST and REISS (2), did not observe this polarity, since it is apparent only when leakage along surface films is small or excluded. Of course it is still possible that the inverse transport of the highest auxin concentrations through 3 and 4 mm. sections was due to some leakage. This does not seem likely, however, considering the long transport time and the sudden onset of the curvature.

With regard to the remarkable differences in the amount of auxin leakage between the older transport experiments with the original technique (8, 6, 9) and these new experiments, something more has to be said. Whenever the auxin moves to a recipient in the form of an agar block, diffusion through tissues or the agar greatly exceeds leakage along the surface. The latter must occur of necessity, since the auxins are surface active; that is to say, they will accumulate in a water-air interphase. How relatively unimportant a movement along this interphase is in competition with diffusion is demonstrated by the value obtained for the diffusion constant of auxin. This was determined by letting the substance diffuse from a thin agar layer into a stack of three other agar layers. The value obtained in this way did not differ greatly from the value expected, on account of the molecular volume, so that the spreading along the agar surface could not have been more than a fraction of the total amount diffusing.

To account for the relative importance of surface leakage in the type of experiments described in this paper, the following fact has to be remembered. Although movement of auxin in an interphase will take place in a monomolecular film, it may be exceedingly fast, provided all auxin arriving at one end is immediately removed (1). This will not be the case if at this end the auxin has to diffuse into agar. But if the arriving auxin is completely removed by the apical cells near the cut surface of the coleoptile (which is quite possible since the auxin transport inside the coleoptile is equally large with, as against, a concentration gradient, 7), a large amount of auxin might be moved in the moist surface film along the epidermis of the coleoptile. Thus the discrepancy in the results might be in the type of receiving material of the auxin, whether agar or living plant cells.

The transport rate experiments with different substances have

been the first to allow a critical test of THIMANN's hypothesis (5) that the activity of a substance in the *Avena* test depends not only on its growth activity per se, but also on its transport rate. Thus substances with a slow rate of transport would seem only slightly active in the *Avena* test. As far as this work goes it has borne out THIMANN's assumption. If the substances tested are placed in order of their molar activity in the *Avena* test, indoleacetic > indolebutyric, anthraceneacetic > naphthaleneacetic > indolepropionic > cis-cinnamic acids, then this series represents the order in which the transport rates of these substances decrease. For the last two it could not be measured at all. This is not surprising, since the curvatures they induce in the *Avena* test are shorter than 4 mm.

At the same time it is apparent that the difference in transport velocity is not the only factor which determines the difference in activity of these substances in the *Avena* test. First there is a difference in the time of onset of the curvature, which is independent of transport velocity. Therefore, this moment of beginning of the curvature must be determined by the rate of penetration of the compound into the cell and/or the rate of the growth reaction proper. Especially such growth promoting substances as have a benzene nucleus only (as phenylacetic acid) have a very slow rate of reaction. In addition, the fact must be considered that the capacity of the transport of growth promoting substances is very limited. This means that if less is transported than is necessary for a growth response no curvature at all will result. This is the case with most growth promoting compounds with a benzene nucleus, which all give S-shaped curvatures in the *Avena* test. Take phenylacetic acid as an example. That it is transported downward is indicated by the positive curvatures which it induces down to about 1 cm. below the cut surface, when an agar block containing a 10^{-4} molar solution is put one-sidedly. When sections are submerged in this concentration they will grow as much as they do in the considerably lower optimal indoleacetic acid concentrations. In the *Avena* test, however, the negative curvature due to the growth promoting properties of phenylacetic acid is limited to a zone of not more than 2 mm. length. These facts can be correlated in the following way: To get equal increases in growth, about a 100× higher concentration of phenyl-

acetic than of indoleacetic acid is required. Since the amount which can be transported inside the *Avena* coleoptile is limited, apparently not enough phenylacetic acid reaches the growing cells farther down to produce a visible growth increase, although the positive curvature indicates that some phenylacetic acid was present. In another paper this curious positive curvature will be considered in greater detail.

Summarizing this discussion, it may be said that the extent of the *Avena* test curvature depends on several different properties of a growth promoting substance: (1) the rate of transport of the substance inside the coleoptile tissue; (2) the rate of growth reaction, indicated by the time of beginning of the growth curvature; (3) the amount transported in connection with the activity of the substance in *Avena* growth. Point 3 must be investigated with more direct methods, but until we have a method which will allow the analysis of extremely small amounts of such phenyl compounds, no direct determinations seem possible.

Summary

1. Adapting the photokymograph recording of the *Avena* test, a new technique for measuring the velocity and capacity of the transport of growth promoting substances through different tissues has been worked out. For this the rate of bending and the moment of beginning of curvatures are compared for a normal *Avena* test and one in which the agar block is separated from the test plant through the tissue of which the transport properties are to be determined. Thus for individual pieces of tissue these properties can be calculated.

2. In general the results of VAN DER WEIJ (6) were confirmed, as far as the transport properties of the *Avena* coleoptile are concerned. Transport velocity is independent of length of tissue, but the amount transported decreases with increased length of coleoptile section. Transport through the narrow or wide side of a coleoptile is practically the same. The lower part of the coleoptile has slightly different transport properties from the more apical zones.

3. The polarity of indoleacetic acid transport is far more pronounced than most of the earlier investigators found. In our experiments leakage along moist surfaces was more nearly excluded. Only

the highest auxin concentrations (1000 mg./l.) were transported from base toward top through 3.1 and 4.2 mm. sections, but not through 6.3 mm. coleoptile sections.

4. There are great differences in transport velocity among various growth promoting substances. Owing to this and to differences in the rate of the growth reaction and limited transport capacity of the coleoptile cells, a wide variation results in the apparent growth activity of these substances in the *Avena* test.

5. Transport of indoleacetic acid through other plant tissues was determined, with positive results in corn coleoptile, papaya leaf stalks and midribs, *Tropaeolum* leaf stalks and flower stalks, and *Nitella* internodal cells.

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CYTOLOGY OF DORMANCY IN PHASEOLUS AND ZEA

FREDERIC G. MIDDENDORF

(WITH NINETEEN FIGURES)

Introduction

Dormancy of plant seeds and spores, as well as the analogous problems in animal life of encystment, desiccation, aestivation, and hibernation, have been a common source of research in the recent past. Excluding the studies on seed coats, however, little cytological work has been done on the dormant seed. The cytology of dormancy in *Zea* has been studied by HICKERNELL (13, 14) and in *Phaseolus* by KATER (16). Both investigators described a migration of chromatin as the important phenomenon. HICKERNELL portrayed a migration of chromatin from the periphery of the dormant nucleus to the center in the germinated or active nucleus of corn and the rotifer; KATER described just the opposite migration in the bean, concluding that the dispersed chromatin in the predormant nucleus migrated via the linin threads into the nucleolus of the dormant cell and returned to the normal dispersal at germination. MACDOUGAL, LONG, and BROWN (18) investigated the cytology of active and desiccated *Echinocactus*, and agreed with HICKERNELL in describing a migration of chromatin from the periphery of the desiccated nucleus to the center of the active one. They state that during recovery, "the granular material that had accumulated in the peripheral portion of the nucleoplasm migrates to the central region of the nucleus" (p. 325).

The initial aims of the present investigation were: (1) to check the previously described cytological basis of dormancy and to determine which of these migrations is correct, or whether there is a migration at all; (2) to establish further, if possible, a more general morphological basis of dormancy.

Material and methods

Phaseolus lunatus var. *lunonanus* (dwarf or baby lima bean), *Zea mays* var. *indentata* (dent corn), and *Pisum sativum* (garden pea)

were the types chosen for observation. As a rule only the hypocotyl of both dormant and germinated seeds were fixed, for the study was concentrated on pith, cortex, and vascular areas of the radicle. For germination, seeds were soaked in warm tap water for several hours until the seed coats were completely expanded, then placed on moist filter paper in petri dishes for about two days at room temperature. Growth was faster and roots were thicker if the seeds were kept on moist filter paper in a germinator at 30° C.

A number of fixations were chosen as likely to give varied results: (1) Karpechenko's chrome-acetic-formalin; (2) Flemming's strong solution, chrome-acetic-osmic; (3) sublimate-acetic-alcohol; (4) Regaud's formol-bichromate; (5) Mann-Kopsch's sublimate-osmic. Sections were cut usually 4 μ thick.

Two stains were employed after each of the fixations except the last: Heidenhain's iron haematoxylin and basic fuchsin for the Feulgen reaction. For the iron haematoxylin stain following the Karpechenko, Flemming, and sublimate fixations, sections were mordanted in 2 per cent iron-alum for five minutes at 40° C., stained in 1 per cent haematoxylin at 40° C. for ten minutes, then destained in 2 per cent iron-alum. Following Regaud's fixation, sections were mordanted in 3 per cent iron-alum for one hour at 37° C., stained for two days in 2 per cent haematoxylin at room temperature, then destained in 3 per cent iron-alum. For the Feulgen reaction, hydrolysis was effected by treating the sections of material fixed in Karpechenko's, Flemming's, or Regaud's with normal HCl at 50° C. for twenty to twenty-five minutes. A five minute hydrolysis was used for sublimate preparations. Hydrolysis was preceded and followed by a two minute treatment in normal HCl at room temperature. Sections were then stained in fuchsin-sulphurous acid for at least three hours and washed in three or more changes of SO₂ water.

Drawings were made with the aid of a camera lucida from a Leitz fluorite objective and a no. 15 compensating eyepiece. With one exception, cells to be illustrated were chosen just above the area of division or what corresponds to that area in the dormant radicle. In fact, attention has been concentrated on this locality during the entire study, because of the obvious activity of these cells during germination.

The following method was employed to determine water content of the dormant radicle. The radicles of a great number of seeds were dissected out, weighed, desiccated in an oven at 70° C. for a week, then reweighed and the percentage of water calculated. The percentage of water of radicles clipped from germinated seeds was determined in the same manner.

Germinated seeds were artificially dried for cytological study in a desiccator overnight at 37° C.

Observations

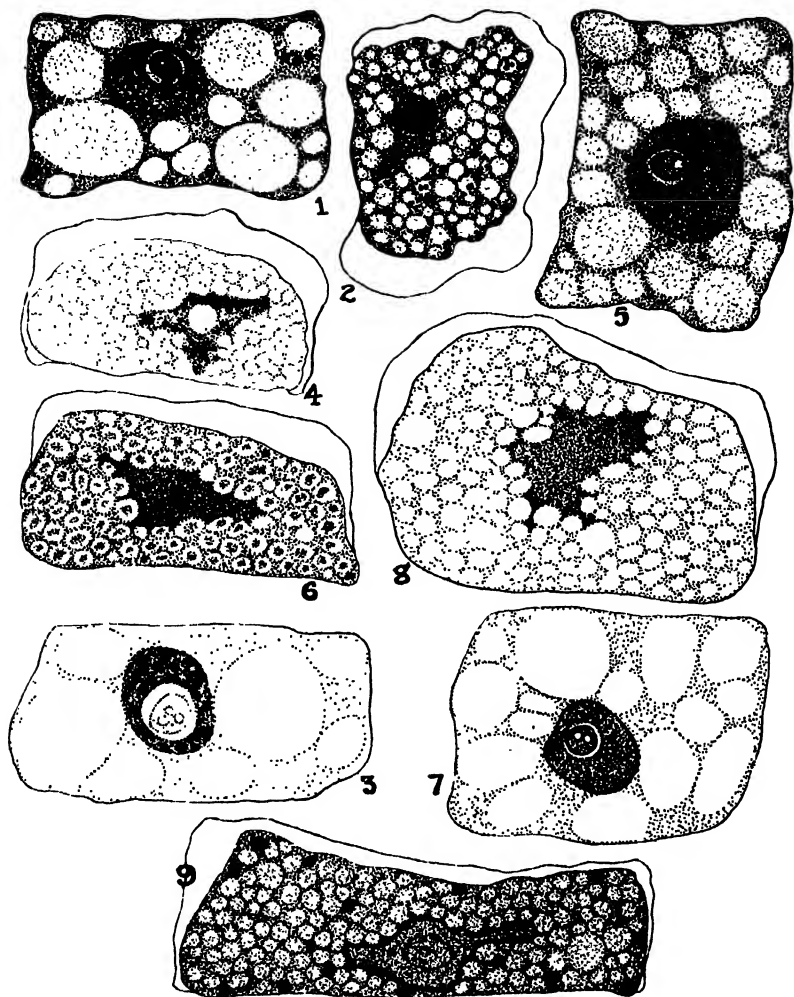
PLASMOLYSIS

Plasmolysis is the most obvious characteristic of the dormant cell. It is strikingly evident in the cotyledon, radicle, and plumule of dormant bean and pea, and in the cotyledon, endosperm, and radicle of dormant corn, but it is almost absent from the plumule of the latter. This is due no doubt to the arrangement of cells in layers two or three rows thick. Possibly when water is lost the whole leaf primordium shrinks instead of the individual protoplast shrinking from the cell wall. The cells of the dormant radicle of the bean, pea, and corn appear characteristically to plasmolyze in pairs facing each other (fig. 19). Artificial desiccation of germinated radicles did not produce plasmolysis. The cell walls shrink with the protoplast, usually distorting or rupturing both cell wall and vacuoles.

NUCLEUS

GERMINATED SEEDS.—The germinated nucleus is turgid and plump; its shape is normally spherical, with a few oval or lobose amoeboid forms (figs. 1, 3, 5, 7, etc.). It is bounded by a definite limiting membrane which seems to be darkly stained by iron haematoxylin (figs. 1, 5). This membrane does not react to the Feulgen test, but is differentiated by prior fixation.

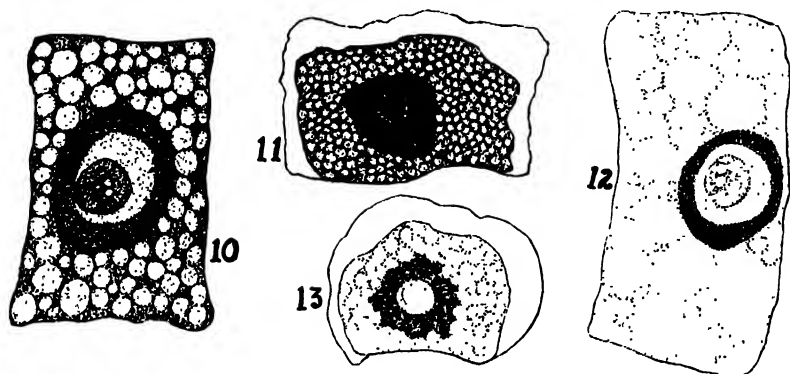
A coarsely granular reticulum is demonstrated by the Feulgen reaction following Karpechenko's (figs. 3, 12), sublimate, and Regaud's fixations. Flemming's fixation gives a finer reticulum (fig. 7). The reticulum of corn stains more brilliantly than that of the bean when both are given the same treatment. This suggests a greater concentration of nucleic acid in corn. Similarly it may be



FIGS. 1-9.—Drawn from cortex of bean radicle with camera lucida, fluorite oil immersion, and $15\times$ compensating ocular. All from 4μ sections; $\times 1966$. Fig. 1, germinated, Karpechenko-haematoxylin. Nucleolus contains a small vacuole. Note plastin granule in perinucleolar area, prominent nuclear membrane, and large vacuoles of cytoplasm. Fig. 2, dormant, Karpechenko-haematoxylin, showing solid black nucleolus with slight perinucleolar area; fine granular reticulum of irregular nucleus does not stain much more intensely than the cytoplasm. Note absence of nuclear membrane and small vacuoles of cytoplasm. Fig. 3, germinated, Karpechenko-Feulgen. Note coarse granular reticulum of oval nucleus. Nucleolus not stained, but differentiated by fixation. Fig. 4, dormant, Karpechenko-Feulgen. Note coarse granular reticulum of stellate nucleus and hyaline center representing nucleolus. Fig. 5, germinated, Flemming-haematoxylin, similar to fig. 1, but nucleus less coarsely granular. Fig. 6, dormant, Flemming-haematoxylin, similar to fig. 2. Nucleolus stained as rest of nucleus; latter readily distinguished from cytoplasm but noticeably lacks a membrane. Fig. 7, germinated, Flemming-Feulgen, showing finer reticulum and smaller perinucleolar zone than fig. 3. Nucleolus stained. Fig. 8, dormant, Flemming-Feulgen, showing finer reticulum than fig. 4. No nucleolus present; other cells normally possessed unstained nucleoli. Fig. 9, dormant, Mann-Kopsch, showing thick structure bounding nucleus after osmication. This membrane appeared hyaline but is represented in black.

concluded that nucleic acid is more concentrated in chromosomes than in the reticulum, for the former stain more intensely than the reticulum of the resting nucleus.

The nucleolus is always evident in germinated seeds, even if not stained. Iron haematoxylin stains it black, usually with a more dense rim or border and a lighter center (fig. 1). This center is normally vacuolated (fig. 10), and the black rim frequently has one or



FIGS. 10-13.—Corn radicle, Karpechenko fixation. Drawn from cortical parenchyma with camera lucida, fluorite oil immersion, and $15\times$ compensating ocular; 4μ sections; $\times 1966$. Fig. 10, germinated, haematoxylin, similar to fig. 1 except for larger perinucleolar zone and smaller vacuoles in cytoplasm. Fig. 11, dormant, haematoxylin, similar to fig. 2 except that nucleus is less irregular and nucleolus does not stain darker than rest of nucleus. Fig. 12, germinated, Feulgen, similar to fig. 3. Fig. 13, dormant, Feulgen, similar to fig. 4.

two jet black spherical granules adjoining it in the perinucleolar area (fig. 1). CARLSON (7) considers this granule of a plastin nature. The Feulgen reaction normally leaves the nucleolus unstained. Some Flemming material, however, shows nucleoli stained (fig. 7); and after Karpechenko's a few nucleoli appear lightly colored, especially around their border. This was also observed by CARLSON following certain fixatives. The preceding fixations differentiate a boundary and an internal vacuolate structure which are just as evident when the nucleolus remains uncolored as when some of the stain is deposited (figs. 3, 12).

The perinucleolar area, usually very evident in germinated seeds, is normally larger in corn than in bean; compare figures 1 and 3 with figures 10 and 12.

DORMANT SEEDS.—Dormant nuclei lack the plumpness of germinated ones; instead they were shrunken to a stellate or crenate form. A comparison of figures 3 and 4 with figures 12 and 13 illustrates that departure from the normal, spherical, nuclear type of the active plant is much more noticeable in the bean than in corn. In the latter, pith, cortex, and vascular areas contain crenated nuclei (fig. 13), among which vascular nuclei are the smallest. In the bean, pith and cortical nuclei are irregularly stellate or of almost any possible shape, the processes wedging between the vacuoles of the cytoplasm (figs. 6, 8). Vascular nuclei are merely crenated as in corn.

No stained or even differentiated limiting structure or membrane can be observed in the dormant nucleus following Karpechenko's (figs. 2, 4, 11, 13), Bouin's, Regaud's, or sublimate fixations. Usually after Mann-Kopsch's fixation a membrane is strikingly evident and exaggerated (fig. 9). Such a membrane also appears occasionally after Flemming's strong, and is probably due to osmication. After the first named fixations, haematoxylin stains the nucleus gray or black, the periphery of which, especially in the bean when sufficiently destained, is scarcely distinguishable from the cytoplasm (fig. 2). The color abruptly fades out or blends with that of the cytoplasm between the vacuoles. The Feulgen reaction likewise shows no structure limiting the dormant nucleus, nor is one differentiated by prior fixation. The invisibility of this supposed membrane after the majority of fixations is significant and indicates its probable absence in the dormant cell. Possibly some conclusion regarding the nature of the nuclear membrane can be drawn from the phenomenon. If the structure disappears upon the loss of water, as it apparently does, it is scarcely a true morphological membrane as stated by HICKERNELL (14) for corn and rotifer, but is more plausibly regarded simply as an interface.

The Feulgen reaction stains a reticulum after the majority of fixations as a cluster of purple-red granules or flakes (figs. 4, 13). After the Flemming fixation the reaction often gives a more even deposition of color (fig. 8). The fact that the reticulum of the cells of the dormant radicle is more intense in its reaction to the Feulgen technique than that of cells of the germinated radicle suggests that chromatin is more concentrated or more compact in the dormant seed.

Although the nucleolus is an outstanding structure of the dormant nucleus stained by haematoxylin, the Feulgen reaction leaves it unstained; compare figures 1 and 4, or figures 11 and 13. In the bean, haematoxylin stains the nucleolus solid black (fig. 2); it is sometimes slightly irregular in outline (fig. 15), but has no internal vacuolate structure as was observed in this organelle in the germinated seed. In dormant corn, the nucleolus may stain more lightly than the reticulum, as described by HICKERNELL (13, 14), but only after a short haematoxylin stain. In this case, too, no internal vacuolate structure is evident (fig. 11). Not only does the Feulgen reaction fail to stain nucleoli, but the preceding fixation also leaves them undifferentiated. However, a spherical, hyaline, central area may be interpreted as representing one (figs. 4, 13).

The perinucleolar area is slight or absent in dormant cells.

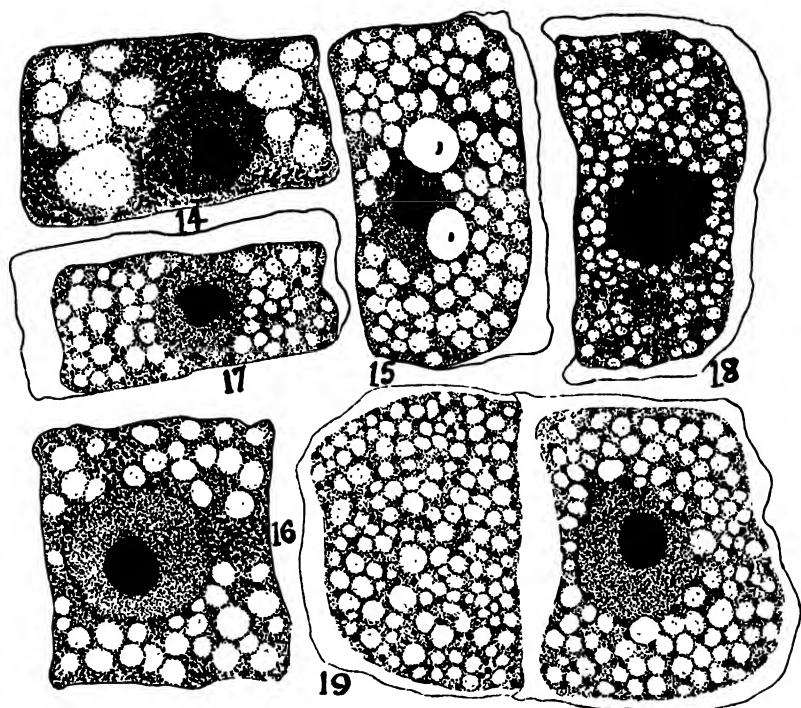
CYTOPLASM

GERMINATED SEEDS.—Vacuoles of the germinated cell vary in size, depending on the position of the cell in the radicle. Cells nearest the root tip are packed with small vacuoles. As the cell becomes farther removed from the root tip the vacuoles gradually enlarge. Cortex and pith of the same region contain vacuoles of similar size which are slightly larger than those of the small vascular cells.

Although it seems advisable to distinguish with SOROKIN (22) between "mitochondria" and "primordia of plastids," if this is possible, such a distinction has not been made in the present work, since the Janus green B test has not yet been applied. Consequently the term mitochondria is used to include both "mitochondria" and "plastid primordia."

In the germinated seed, mitochondria are crowded between the vacuoles (figs. 14, 16). They may be rather evenly distributed throughout the cell or concentrated around the nucleus. One bean radicle possessed solely long filaments, spiral or horseshoe-shaped or with ends attached to form a ring. But the normal mitochondrial complex of germinated seeds consists of rods with some filaments, granules, and hollow spheres. This agrees with descriptions of N. H. COWDRY (9) for the pea, and ANDERSON (1) for the hyacinth. Bean mitochondria are somewhat larger than those of corn as fixed by Regaud's method; compare figures 14 and 16.

DORMANT SEEDS.—Vacuoles of the dormant seed vary slightly in size within a single cell, but are universally smaller and more numerous than those of germinated seeds. Perhaps this could be inter-



FIGS. 14-19. —Drawn with camera lucida, fluorite oil immersion, and compensating ocular 15 \times . All from 4 μ sections; $\times 2200$; Regaud's method for mitochondria. Some mitochondria drawn above the nucleus. With exception of fig. 18, all taken from cortex just above dividing area (or its equivalent in dormant seed). Fig. 14, germinated bean showing mitochondria as rods. Fig. 15, dormant bean showing mitochondria as granules. Two starch grains evident with hyla stained black. Fig. 16, germinated corn showing mitochondria as short thin rods. Fig. 17, dormant corn showing granular mitochondria. Fig. 18, dormant pea, taken from tip of hypocotyl and showing granular mitochondria in groups. Fig. 19, taken from cortex of same embryo as fig. 18 but showing granular mitochondria uniformly distributed in cytoplasm. Note how cells plasmolyze in pairs.

preted as indicating that a multiplication of vacuoles is associated with dormancy. The tonoblasts or boundaries of the vacuoles remain just as evident in the dormant cell as in the germinated one. Further, dormant vacuoles do not lose their spherical or oval shape as does the nucleus.

I had intended to confine this study to a comparison of the bean and corn, but results obtained appeared to contradict a statement of E. V. COWDRY (8) regarding the mitochondria of the dormant pea. He states: "it has been found by N. H. COWDRY that they [mitochondria] are filamentous in the rather inactive cells of the dried seed pea" (p. 315). Sections of the dormant garden pea were then made, and the mitochondrial type of the pea was found to agree perfectly with that of the dormant bean and corn.

In the dormant cell mitochondria again are crowded between the vacuoles (figs. 15, 17, 19). As for germinated seeds, so for dormant seeds, mitochondria may be evenly scattered throughout the cell or

TABLE 1
CYTOLOGICAL DIFFERENCES BETWEEN DORMANT AND
GERMINATED SEEDS

	DORMANT SEEDS	GERMINATED SEEDS
Plasmolysis.	Present	Absent
Shape of nucleus.	Shrunk, irregular, stellate	Turgid, regular, spherical
Nuclear membrane	Not visible	Visible
Nucleolus.	Not differentiated	Differentiated
Perinucleolar area.	Small or none	Large
Vacuoles.	Small	Large
Mitochondria.	Small spheres	Rods, spheres, and filaments

concentrated in groups around or away from the nucleus. Usually the cells in one area all possess a similar distribution of mitochondria. Such a region may embrace pith, cortex, and vascular areas. Figure 18 shows grouped mitochondria in a cortical cell near the root tip of the dormant pea. Cells some distance above this point in the same hypocotyl possessed an even distribution of mitochondria (fig. 19). Dormant mitochondria are all of the small granular type, with a very few short shrunken rods. With Regaud's method the granules are usually not perfect spheres but pebble-shaped or shrunken. Pea and bean are almost identical in size, shape, and number of mitochondria—about one hundred per cell per $4\ \mu$ section. Mitochondria of corn are very similar but somewhat smaller.

Two large starch grains appear in figure 15. If the hylum is present in the section, it is stained prominently by haematoxylin as

shown in the drawing. In the present work, however, the distribution of starch in bean and corn was not studied. Its distribution in the hypocotyl and plumule of the predormant, dormant, and germinated bean has been described by KATER (16).

The characteristic cytological differences between the germinated and dormant seeds thus far described have been summarized in table 1.

The water weight of the bean and corn were determined and found to be 6.015 per cent of the dormant bean hypocotyl, 2.22 per cent of the dormant corn hypocotyl, 91.86 per cent of the germinated bean radicle, and 89.31 per cent of the germinated corn radicle. It will be noticed that the bean possesses a larger percentage of water than corn in both the dormant and germinated states, but that during germination the corn acquires a larger percentage of water (87.09 per cent) than does the bean (85.85 per cent).

Discussion

Since its publication by FEULGEN and ROSSENBECK (12), the nucleal reaction has been applied to almost every type of cytological material. Literature in regard to the Feulgen reaction is considerable and contradictions not uncommon. For instance, FEULGEN and ROSSENBECK (12) first claimed that yeast gives absolutely no reaction; subsequent observations of MARGOLENA (19), PETTER (20), ROCHLIN (21), and others have shown that this important biological group may give a very intense reaction. A positive reaction for Nissl bodies was reported and denied (for literature see 4 and 23). Further, statements regarding the reaction given by eggs in various stages do not agree. FEULGEN (11) reported unripe eggs and fertilized ripe ones to give a positive reaction, unfertilized ripe eggs to give no reaction. BAUER (2) states that developing eggs of most animals give no reaction. To mention but two recent American workers, BLUNT (5) reports a positive reaction given by nucleoli (net-knots) of early oocytes in *Cambarus*, whereas JOHNSON (15) reports a negative reaction for the entire nucleus of crayfish eggs during the growth stage. It has become a commonplace to pay tribute to the specificity of the reaction for chromatin; but incongruous statements

such as those referred to indicate that an accurate interpretation of the Feulgen reaction demands experience and checking.

The type of fixation modifies the reaction somewhat; one fixation demands a longer or shorter time of hydrolysis (2) and shows a coarser or finer reticulum (23) than another. Hydrolysis weakens the absolute validity of morphological conclusions based on the reaction, for, besides distorting the tissue, it may dissolve the nucleic acid and deposit it in a nearby area of the cell, as observed by BERG (4) and CARLSON (7). The reaction in these instances would not be locally specific. Further, cell elements or products, such as starch and cellulose, may stain with the reaction (3, 7, 19).

In this work several fixations were used as checks on one another. Two of these, chrome-osmic and chrome-formalin, are reported by BAUER (2), who tested for the optimum time of hydrolysis following twenty-four different fixations, as completely resisting distortion by hydrolysis. Study of a great number of slides of material prepared from different fixations indicated that only occasionally did color appear in or on the border of the nucleolus. With the following exceptions, no extra-nuclear structures were observed stained by the reaction: (a) Cell walls of xylem elements of germinated seeds were occasionally stained intensely after any fixation; cell walls of non-vascular tissues of both germinated and dormant seeds rarely stained except after chrome-osmic. (b) Starch grains stained deeply after chrome-osmic, and in one set of slides after sublimate-alcohol fixation. No starch grains were observed stained after chrome-formalin and formol-bichromate. The staining of these structures, however, scarcely offers occasion for confusion with nuclear material, by reason of their form and position. They also give the reaction after a period of hydrolysis insufficient to produce color in the nucleus. In fact, according to MARGOLENA (19), they react without any prior hydrolysis.

Once the loopholes in the validity of the reaction are checked, the Feulgen reaction can be relied on as a specific microchemical test for nucleic acid indicating both the presence and the morphology of chromatin in the cell.

Previous investigators studying the cytology of dormancy have stressed a migration of chromatin as the basic morphological con-

comitant of latency. Although agreeing on this general point, interpretations have differed greatly in specific detail, HICKERNELL (13, 14) believing the movement to be centrifugal; KATER (16) regarding it as centripetal. This disagreement alone might cast suspicion upon the validity of the techniques employed; and after the present study of Feulgen material it is possible to reinterpret the role played by chromatin in dormancy of seeds. In brief, these opposite descriptions of chromatin migration must be discarded in their entirety.

In the first place, the reaction shows no striking difference in the distribution of chromatin of the dormant and germinated seeds. The fact that the stain is somewhat more intense in the dormant than in the germinated nucleus may indicate that chromatin, which we identify with nucleic acid, is more concentrated or more compact in the dormant seed. Probably, however, there is not a greater amount of nucleic acid in the entire dormant seed. It would seem rather that the same amount is crowded into a smaller space in the dormant nucleus because of shrinkage. But apart from this, chromatin or the reticulum scarcely offers a source of cytological difference between dormant and germinated seeds.

In the second place, dormant and germinated nucleoli do not appear to differ in kind; both react negatively to the Feulgen technique and seem to be of the nature of a plasmosome. Others have repeatedly observed that the Feulgen reaction does not stain plant nucleoli (2, 4, 7, 19, 24, *et al.*). In the present work, dormant nucleoli never stained. In the germinated seeds they occasionally stained or showed a light color around the border, but only after the use of a few fixatives. CARLSON made a similar observation and decided that chromatin dissolved by hydrolysis accumulated there. This conclusion seems more sound than any other that suggests itself. Chromatin of the dormant nucleus is probably less soluble, and hence does not accumulate in or near the nucleolus. We may reasonably state that chromatin is not present in either the dormant or the germinated plant nucleolus. All possibility, therefore, of a migration of chromatin from the periphery to the nucleolus or vice versa is excluded. Even though we recognize the possibility that an aldehyde group may be introduced by fixation into a constituent of the nucleolus and so allow for the positive reaction of some nucleoli, it

still would scarcely indicate the presence of nucleic acid, but rather a reaction similar to that of some fixations and starch.

KATER's interpretation grew out of his error in regarding the nucleolus and perinucleolar zone as constituting the entire dormant nucleus and in disregarding the very lightly staining chromatin outside the perinucleolar zone. This mistake is easily made in well destained slides since the dormant nucleus lacks a visible membrane and the boundary between nucleus and cytoplasm is often determined with difficulty, especially after the Bouin or Karpechenko fixations. The significance of HICKERNELL's descriptions I cannot state, but it is certain that there is not a migration of chromatin in the dormant bean and corn, or the Feulgen reaction would show it.

All the described cytological differences between the dormant and germinated seed would seem to be correlated with water content. Obviously plasmolysis and perhaps the shrunken irregular shape of the dormant nucleus and the concomitant loss of a visible nuclear membrane and other internal nuclear changes result from loss of water. Recently KATER (17) correlated the size and shape of mitochondria with the water content of the liver in the rabbit. Increased percentage of water in the liver caused enlargement and enspherulation of mitochondria. In the plant the great increase of water content in the germinated seed is accompanied by enlargement of mitochondria, but the characteristic morphological types are rods and filaments rather than spheres. The fact that both bean and corn show the variation from granules in the dormant to enlarged rods in the germinated seed suggests constancy for the phenomenon in the plant. Water probably influences the size of mitochondria directly but their shape only indirectly.

Summary

1. The cellular morphology of the dormant and the germinated seed was compared in both the bean and corn. Seeds were fixed in various fluids, and after each type of fixation were stained by both iron haematoxylin and the Feulgen reaction.

2. In the dormant state the cell is plasmolyzed; the nucleus has an irregular form, apparently lacking a membrane or limiting structure; the nucleolus is not optically differentiated by fixation although

it may stain intensely with iron haematoxylin. The vacuoles are small, and the mitochondria are small granules or spheres.

3. At germination the cell becomes turgid; the nucleus regains a normal spherical form and the boundary becomes definite; the nucleolus becomes optically differentiated even when unstained as by the Feulgen reaction, and shows a vacuolate structure. The vacuoles of the cytoplasm enlarge, and the mitochondria elongate into rods or filaments.

4. Prior concepts regarding the chromatin of the dormant cell as having migrated centrifugally (HICKERNELL) or centripetally (KATER) are rejected on the basis of the Feulgen reaction.

I am indebted to Dr. J. McA. KATER for constructive criticism throughout the work as well as for the use of his slides of seeds fixed in Bouin's and stained with iron haematoxylin. Thanks are also due to Dr. R. A. MUTKOWSKI for assistance in the preparation of the manuscript.

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EFFECTS OF INDOLEACETIC AND NAPHTHYL- ACETIC ACIDS ON DEVELOPMENT OF BUDS AND ROOTS IN HORSERADISH¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 497

ROBERT C. LINDNER

(WITH FOURTEEN FIGURES)

Introduction

In recent years a number of investigators have emphasized the influence of auxins and similar compounds on the growth processes of plants (4, 25). It was observed that the application of relatively high concentrations of these substances stimulates the production of numerous adventitious roots from stems and other above-ground portions of various plants (1, 3, 6, 7, 9, 10, 11, 13, 15, 22). The stimulation in lateral root production from aerial roots has also been reported (19). It then became of interest to determine the response of a fleshy root to applications of various auxins at relatively high concentrations. The fleshy root of horseradish, *Cochlearia armoracia*, was chosen for this investigation because it produces both roots and shoots readily when cut into transverse segments and placed in a favorable environment. This plant also permits the study of the effects of certain growth influencing substances on the production of buds as well as roots.

Material and methods

The horseradish roots were obtained from a market in Chicago during November, 1937, and stored in a cool basement until used. In most of the work the roots were cut into cylindrical segments about 4 cm. long, and a small notch was made in the lower face of each segment to distinguish the morphologically lower end. The extreme upper and lower segments were usually discarded, those from the central portion being used for the experimental purposes.

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More than 1200 such pieces of root, representing derivatives from more than 300 plants, were used in the course of the following treatments:

1. Saturated aqueous solutions of indoleacetic acid or naphthylacetic acid applied with a brush to the upper, lower, or both cut surfaces.
2. Two per cent mixtures of indoleacetic or naphthylacetic acids in lanolin smeared on upper, lower, or both cut surfaces.
3. Pure lanolin smeared on upper, lower, or both cut surfaces.
4. Two per cent mixtures of indoleacetic or naphthylacetic acid in lanolin applied as a band (about 5 mm. wide) around the peripheral face of the segments near the upper cut surfaces.
5. Untreated.

The segments treated with pure lanolin will be considered the controls, since they responded in a manner similar to the untreated ones. The sections were placed in moist chambers, moist sand, or moist sphagnum moss, and about 2 weeks later the amount of root and bud formation was observed. All experiments were duplicated with root segments placed in running tap water for 24 hours before treatment.

Some roots were split longitudinally down the middle, and to one cut surface was applied pure lanolin, and to the other 2 per cent mixtures of either indoleacetic or naphthylacetic acid in lanolin.

For histological study, material was obtained from segments whose upper cut surfaces had been treated with 2 per cent naphthylacetic acid in lanolin. The response to indoleacetic acid was essentially the same as that for naphthylacetic acid, except that it was less intense, and will therefore not be described. Segments similarly treated with pure lanolin were used as controls. Daily samples were taken. The material was fixed in Navashin's solution, and run through an n-butyl alcohol series into paraffin. Sections were cut at 8, 10, and 12 μ .

Gross observations

The segments which were untreated or treated with pure lanolin produced roots and shoots from the lateral surfaces in the region of the lateral root traces. Shoots were produced near the morphological top of the segment and roots near the morphological bottom,

regardless of the orientation of a segment during the regeneration period. Buds were formed from the morphologically upper cut surface only in association with a lateral root trace. Roots were occasionally formed from the lower cut surface that were not in association with a root trace.



FIG. 1.—Segments of horseradish root, about 4 cm. long, 3 weeks after treatment. Upper surface on left was smeared with 2% naphthylacetic acid in lanolin, while the other segment was treated with pure lanolin. Maintained in moist chamber at about 25° C.

In all cases the application of growth substances induced a striking increase in the quantity of roots and a decrease in the number of buds, and in some instances complete inhibition of the latter (fig. 1). In addition to the numerous roots from the lateral surfaces, the treated segments produced a number of roots at the morphologically lower cut surface, most of them from the cambial region and the phloem. Another effect was a greater formation of callus from the treated surfaces. In the region of peripheral application, growth substances induced the formation of roots that were not in association

with root traces. Table 1 indicates the results of a typical experiment.

When the extreme lower segment of a root was used, it reacted like the others. On the other hand, the uppermost one always produced more roots and buds than any of the other pieces. This upper portion is composed of the stem and the juncture of the stem and root, while the rest of the segments consist only of root.

TABLE 1
EFFECTS OF 2 PER CENT LANOLIN MIXTURES OF INDOLEACETIC
ACID AND NAPHTHYLACETIC ACID ON TRANSVERSE
SEGMENTS OF HORSERADISH ROOT

TREATMENT	NO. OF SEGMENTS	TOTAL NO. OF ROOTS	TOTAL NO. OF BUDS	AV. NO. OF ROOTS PER SEGMENT*	AV. NO. BUDS PER SEGMENT*
Lanolin on bottom.....	24	434	286	18 ± 4	12 ± 2
Lanolin on top.....	73	1416	789	20 ± 4	11 ± 2
Indoleacetic acid on side..	52	1220	324	24 ± 5	6 ± 1
Indoleacetic acid on bot- tom.....	53	1290	310	25 ± 4	6 ± 1
Indoleacetic acid on top..	51	2090	109	42 ± 10	2 ± 1
Naphthylacetic acid on bottom.....	44	2410	43	50 ± 10	1 ± 1
Naphthylacetic acid on side.....	47	3175	0	70 ± 14	0
Naphthylacetic acid on top.....	42	3985	0	102 ± 16	0

* Standard deviation of mean = $\sqrt{\frac{\sum d^2}{n-1}}$.

Washing the segments for 24 hours in tap water before treatment inhibited the formation of callus and resulted in greater invasion by micro-organisms. Aside from these effects, the segments responded similarly to the unwashed ones.

When the entire fleshy axis of a root was split longitudinally and the cut surfaces treated with pure lanolin, there resulted only a small amount of root and bud development. The majority of roots were produced from the stem portion of the axis, while the buds, although fewer in number, were more or less evenly spaced over the entire axis. Application of the growth substances, however, induced the formation of numerous roots from traces in the stem region, and a few from root traces in the extreme lower portion of the root. The

petioles of bractlike leaves growing from the upper portion of the stem part of the axis responded to the treatment by producing roots at their bases.

Water, ethyl alcohol, acetone, ether, and petroleum ether extracts of leaves, buds, roots, and xylem (of the root) were applied in mixtures with lanolin to the cut surfaces of 4 cm. segments, to determine whether the production of shoots could be stimulated. No positive results were obtained with any of these extracts.

Histological observations

GENERAL ANATOMY OF FLESHY AXIS.—The fleshy portion of the horseradish plant is composed mainly of root, but a small amount of stem and the junction of the root and stem compose the upper part. Development of the root is somewhat similar to that of the ordinary radish, described by HAYWARD (12). The primary tissues of the root develop from three apical histogens. The primary vascular tissues may be diarch to pentarch. Numerous air spaces are found in the cortex, close to the root tip (fig. 2). The pericycle is at first one-layered, but it becomes active just prior to cambium formation and forms a multi-layered region. The outer layer develops a phellogen, the activity of which later results in the rupturing and breaking off of the entire cortex (fig. 3). Radial divisions of the phellogen and its derivatives, as well as derivatives of the pericycle and secondary phloem, keep up with the increase in diameter of the root which results from the activity of the cambium (fig. 4). The mature root is composed primarily of secondary phloem and secondary xylem parenchyma cells filled with starch (fig. 5). The xylem constitutes the bulk of the root, and comparatively large vessels are scattered among the parenchymatous elements. Small strands of phloem, derived from proliferations of secondary xylem parenchyma cells, are scattered throughout the central portion of the xylem. It is difficult to determine whether any pericyclic derivatives remain in an old root, since the peripheral tissues are more or less homogeneous in appearance and are continuous with the secondary phloem. In some old roots there undoubtedly remain some cells derived from the original pericycle. Elongated stone cells are found scattered throughout the outer portions of the phloem, and the

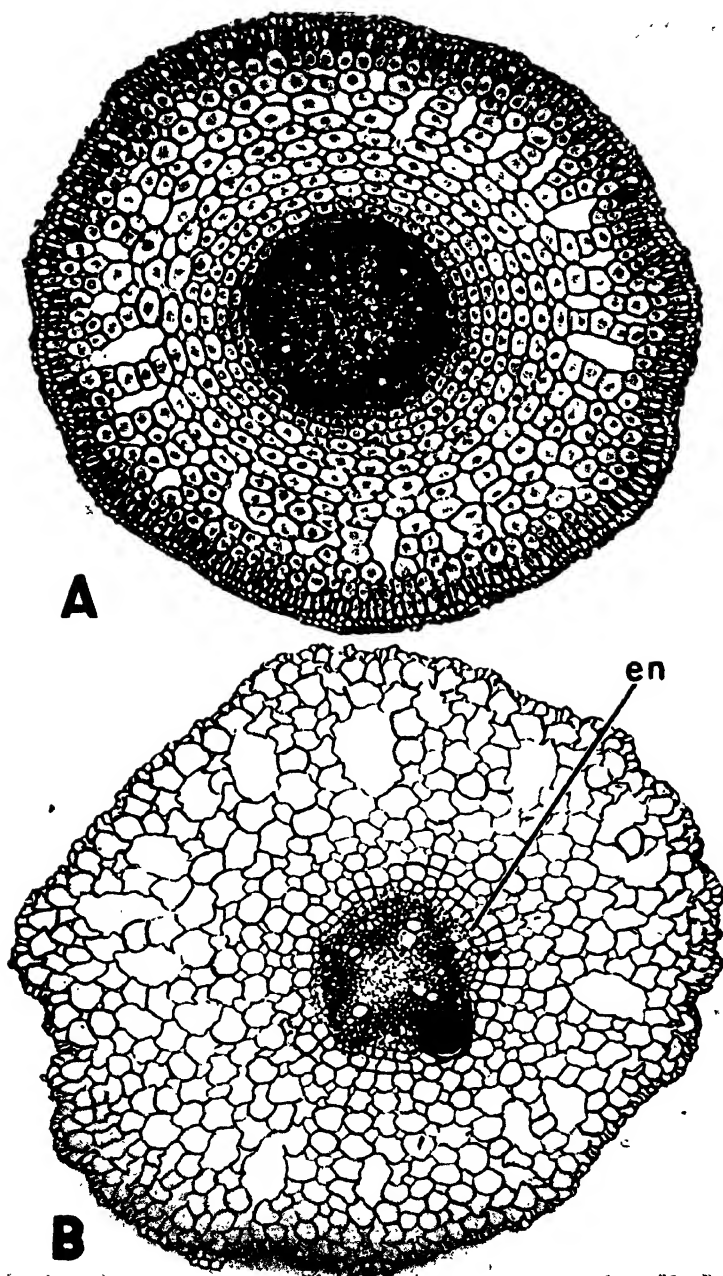


FIG. 2.—*A*: 2 mm. from tip; numerous air spaces in cortex. *B*: 15 mm. from tip; air spaces larger. Lateral root from pericycle. *en*, endodermis.

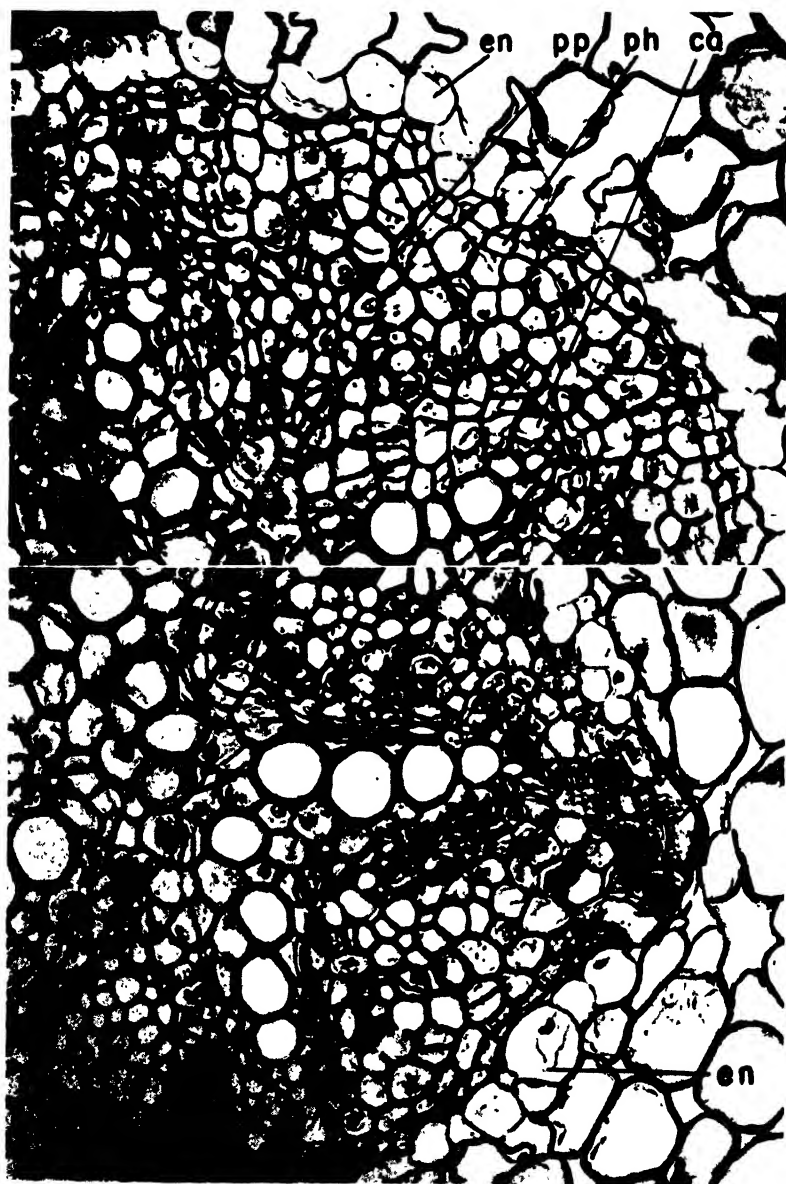


FIG. 3.—Young roots showing beginnings of secondary activity. *A*: parenchymatous derivatives of formerly one-layered pericycle form a band about five cells wide; outer layer of these cells is developing into a phellogen; cambium being formed in parenchymatous cells between primary phloem and xylem. *B*: younger than *A*. Lateral root primordium forming from pericyclic derivatives and crushing endodermis. *en*, endodermis; *ca*, cambium; *ph*, phellogen; *pp*, primary phloem.

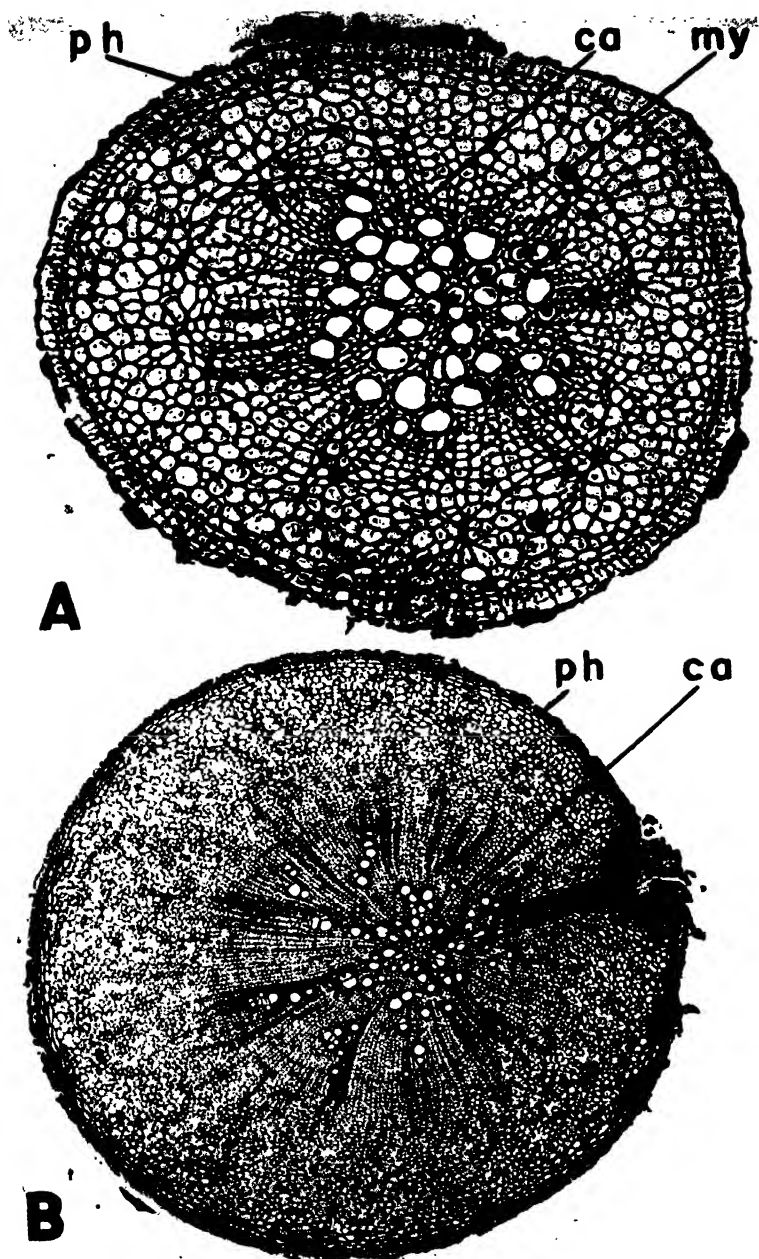


FIG. 4.—Young roots showing secondary activity. *A*: about 1 mm. in diameter. Periderm derived from pericyclic derivatives; cortex absent. *B*: older root, about 3 mm. in diameter, with lateral root trace, and having all structures characteristic of roots 20–30 mm. in diameter, except intraxylary phloem. *ph*, phellogen; *ca*, cambium; *my*, myrosin storage cell.

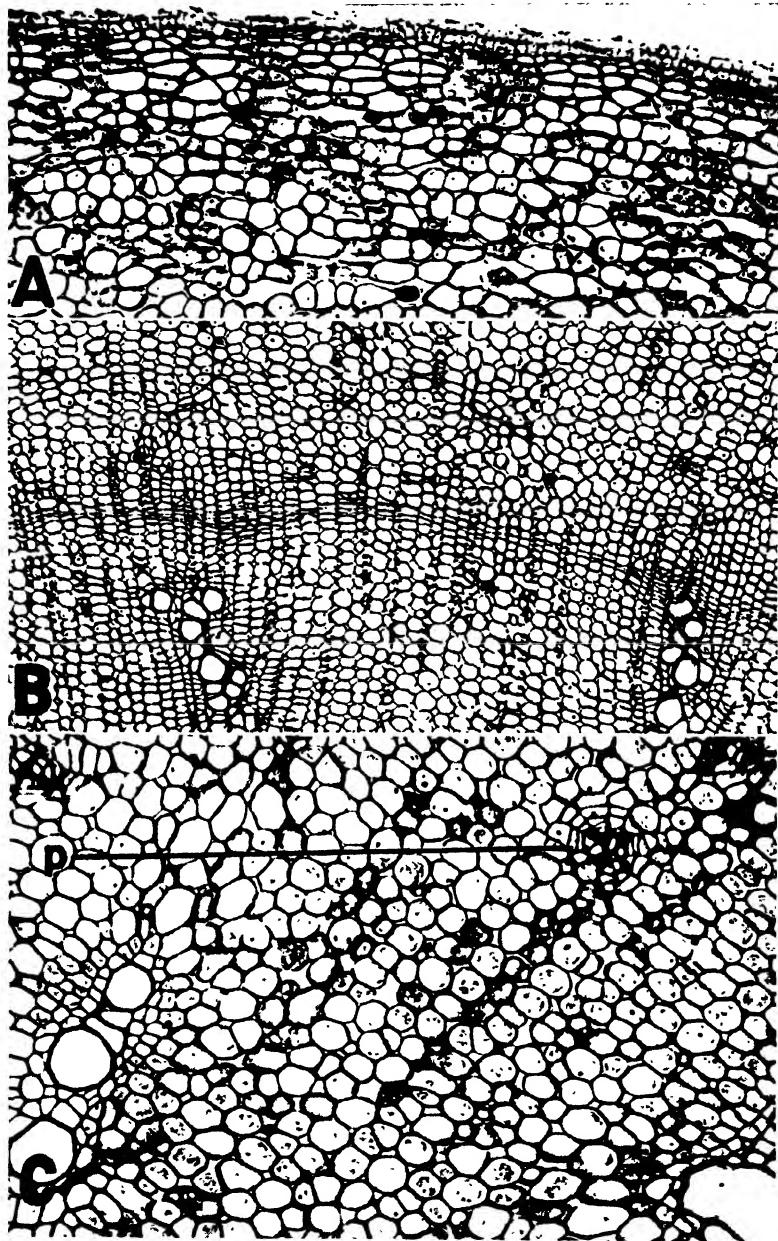


FIG. 5.—Cross sections of portions of roots about 30 mm. in diameter. *A*: periderm and outer phloem. *B*: cambium with associated secondary phloem and xylem. *C*: secondary xylem with strands of intraxylary phloem; parenchymatous cells filled with starch. *p*, phloem strand.

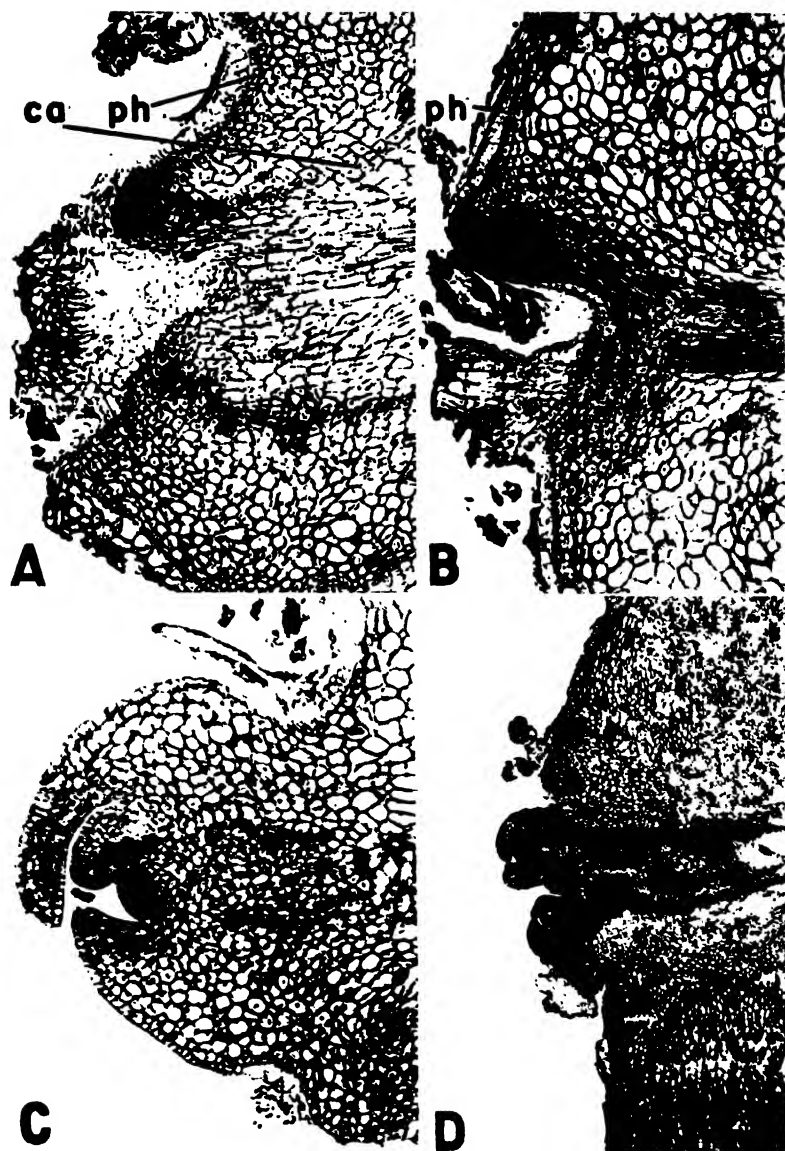


FIG. 6.—Development of buds near lateral root traces of untreated segments. *A*: 4 days old; initial activity involving derivatives of phellogen and cambium of root trace. *B*: 6 days old with one bud developing. *C*: 8 days; developing bud has ruptured periderm; vascular differentiation beginning. *D*: 10 days; vascular tissues of buds connected with those of root trace. *ca*, cambium; *ph*, phellogen.



FIG. 7.—Development of roots in treated segments near lateral root traces. *A*: 4 days after treatment; initial stages involving derivatives of phellogen and cambium of lateral root trace (*cf.* fig. 6*A*). *B*: 8 days after treatment. Emergence of young root through periderm (*cf.* fig. 6*C*). *C*: 10 days after treatment, showing well established vascular connections (*cf.* fig. 6*D*). *ca*, cambium; *ph*, phellogen.

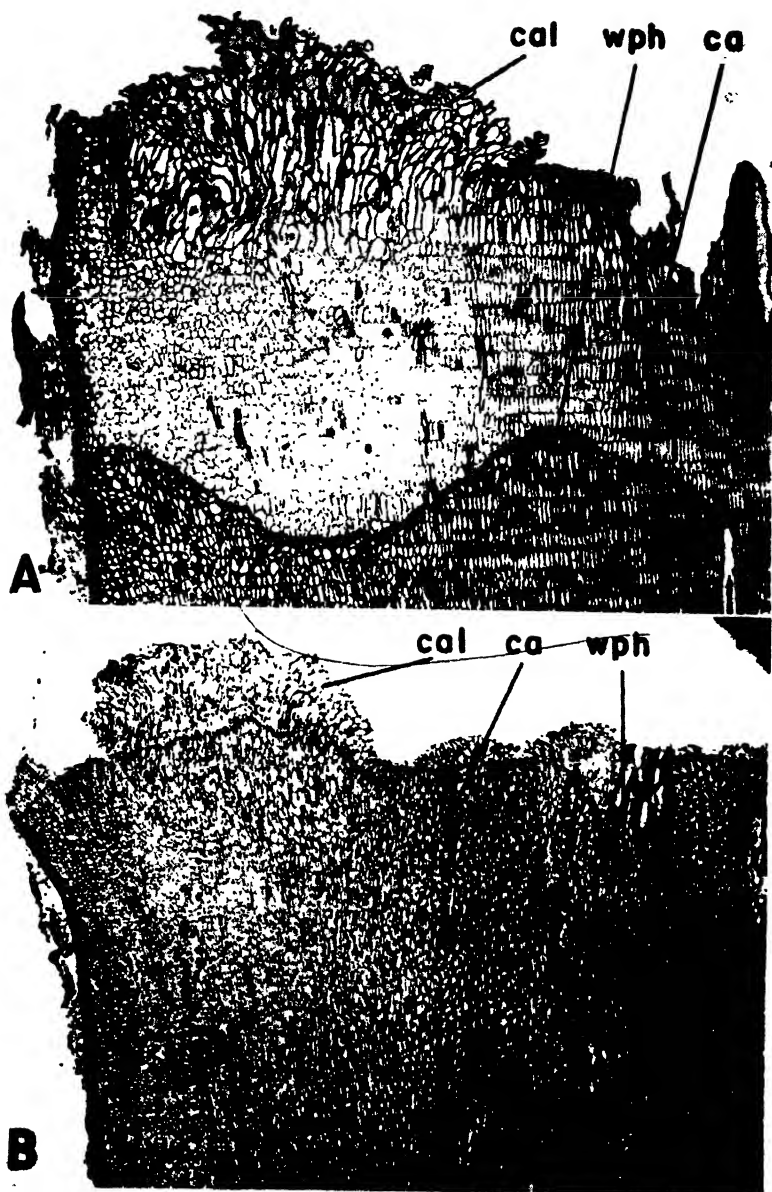


FIG. 8.—Sections through upper cut surface of segments 13 days after treatment. *A*: control. Many cells of phloem and xylem have died; phellogen has formed deep in the phloem and xylem. *B*: treated. Cells have died to considerable depth in xylem; wound phellogen forming; very few dead cells in phloem. *wph*, wound phellogen; *ca*, cambium; *cal*, callus.

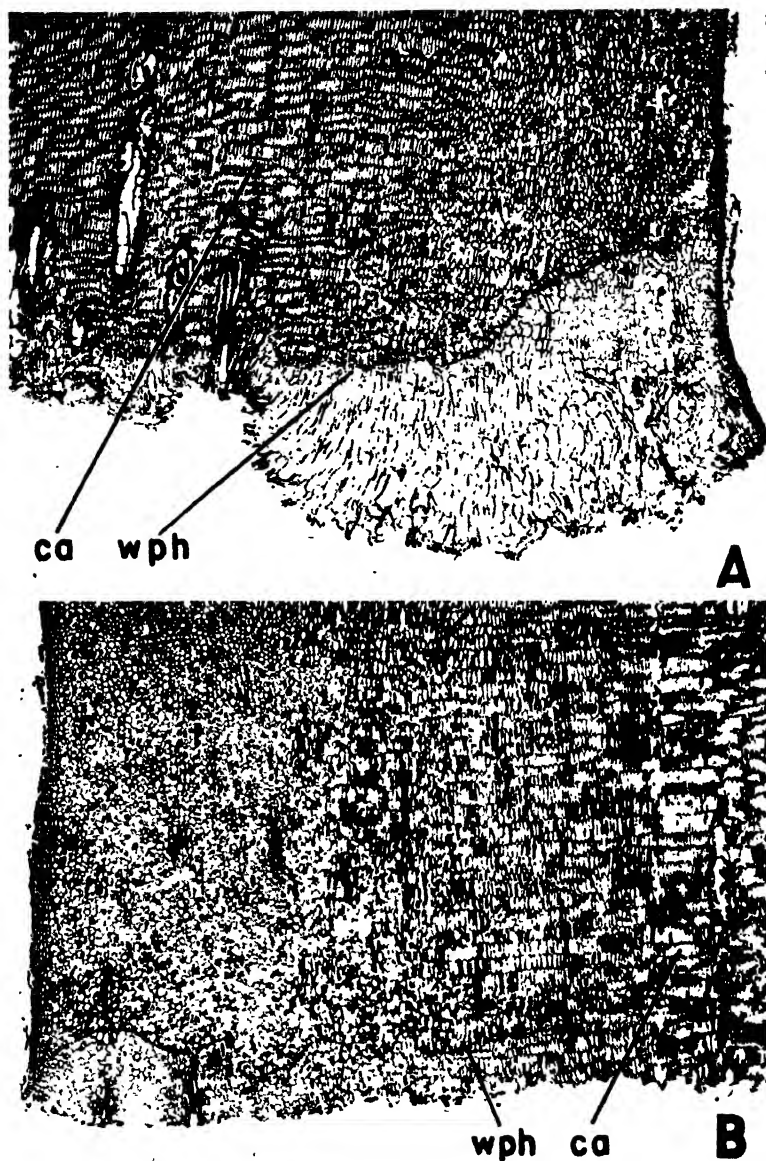


FIG. 9.—Sections through lower cut surface of segments 6 days after treatment. *A*: control. Phellogen is forming that will isolate the calluses. *B*: treated. Very little activity leading to callus formation; phellogen forming. *wph*, wound phellogen; *ca*, cambium.

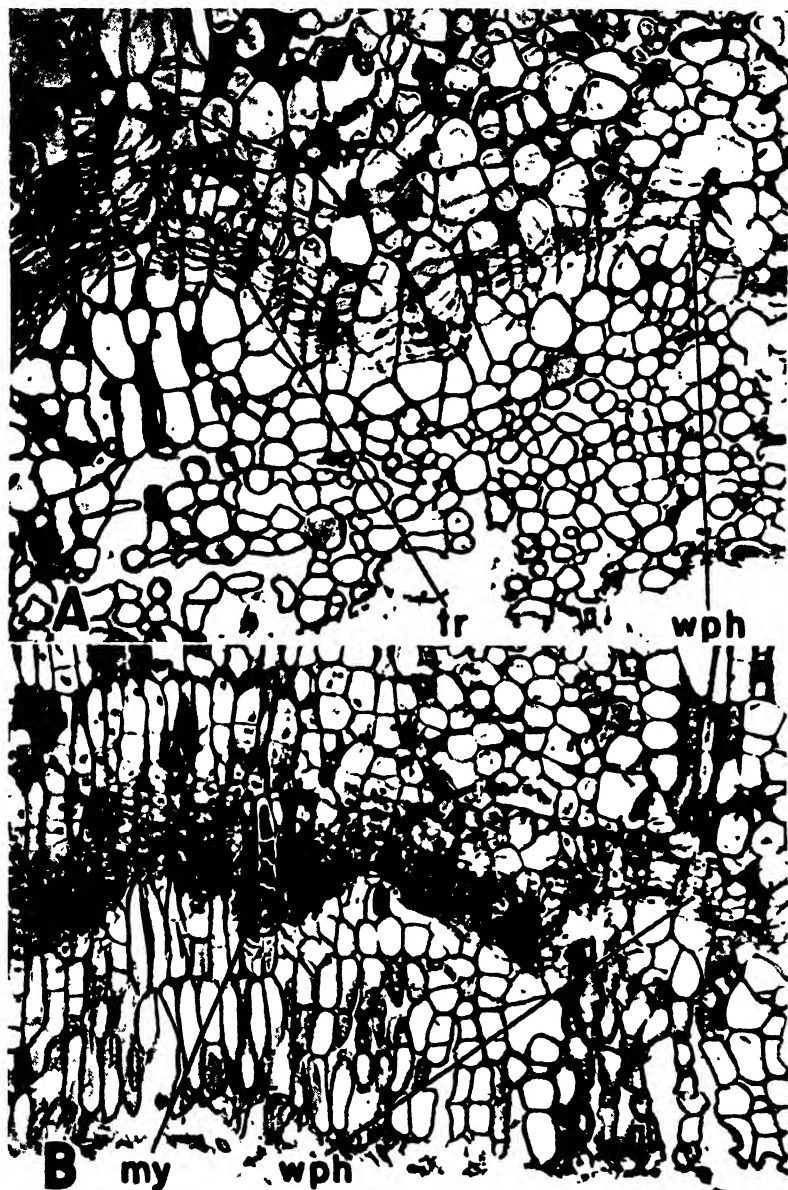


FIG. 10.—Sections through phloem region of lower cut surface of treated segment, 8 days after treatment. *A*: group of isolated tracheids developing from derivatives of secondary phloem parenchyma. *B*: early stage in root formation. Derivatives of wound phellogen and associated secondary phloem parenchyma cells involved. Myrosin storage cell (*my*) has undergone several divisions. *tr*, tracheid; *wph*, wound phellogen.

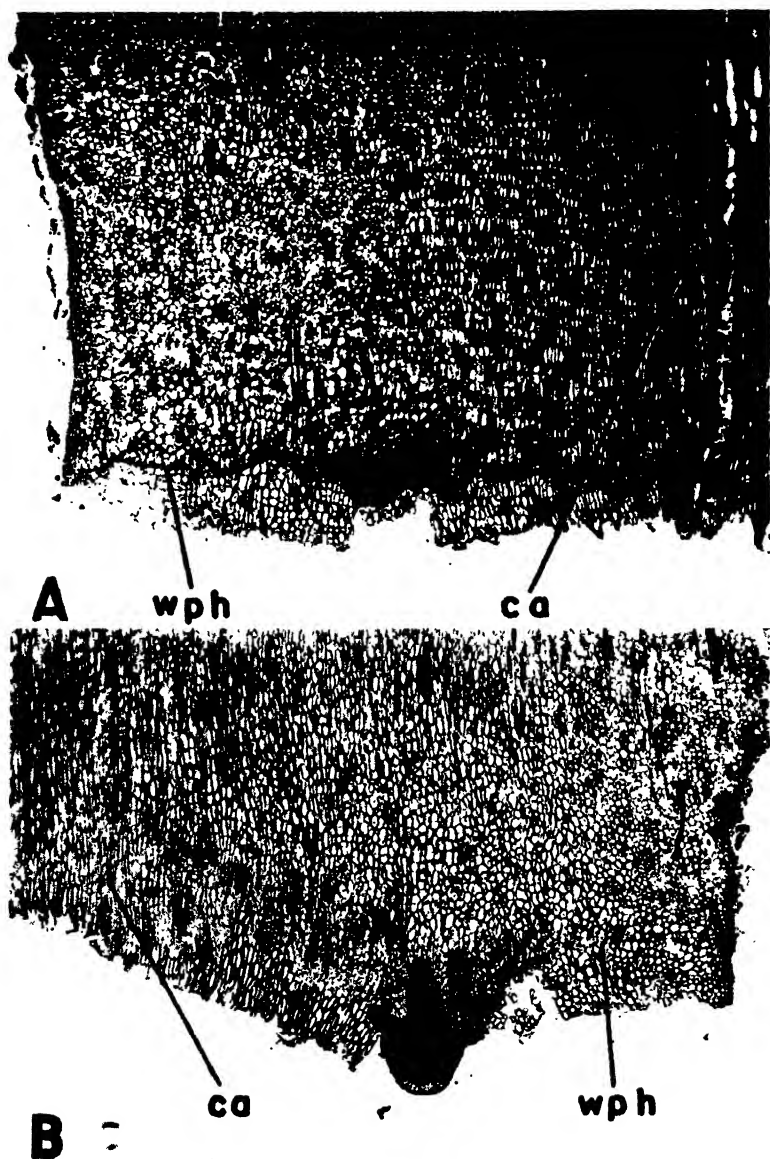
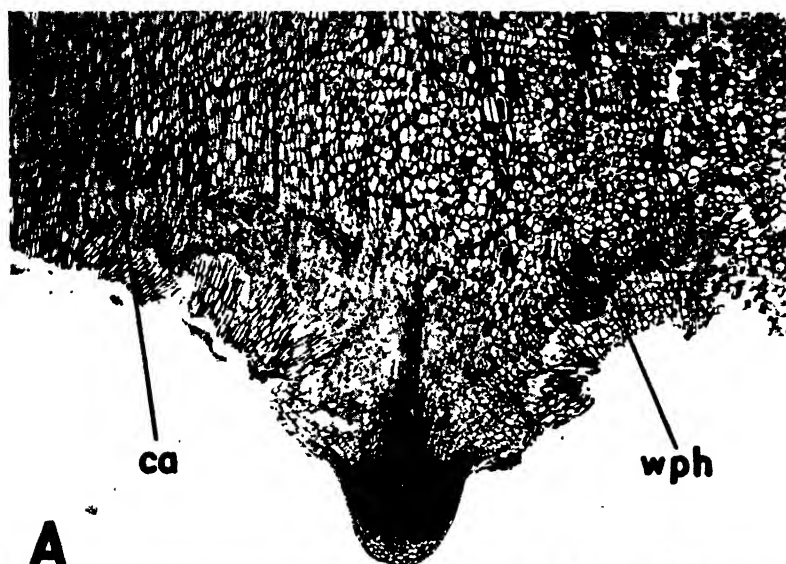
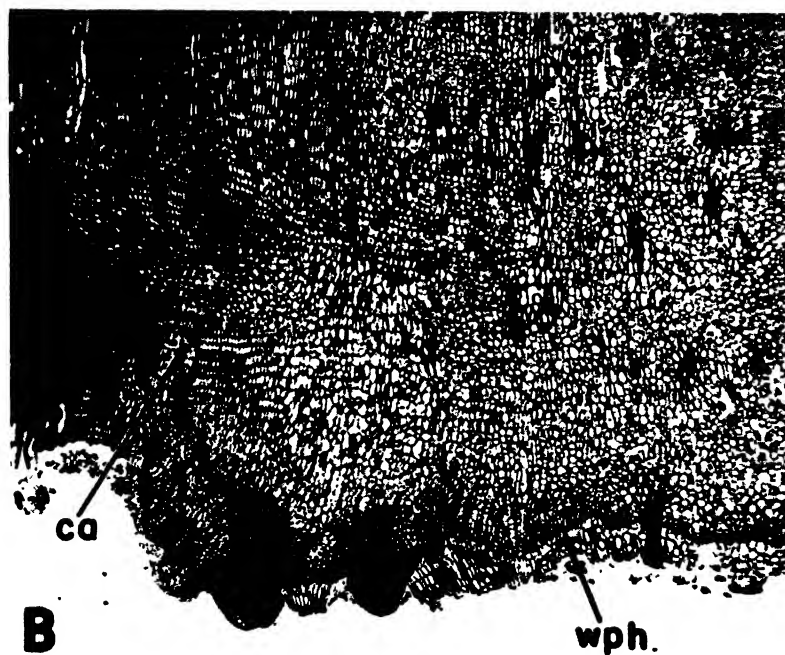


FIG. 11.—Sections through lower cut surface of treated segments. *A*: 10 days after treatment. Secondary phloem parenchyma deeper in from cut surface becoming involved in formation of root primordia. *B*: 13 days after treatment. A root is becoming organized and some cells between developing root and cambium are differentiating into vascular elements. *ca*, cambium; *wph*, wound phellogen.



A



B

FIG. 12.—Sections through lower cut surface of segment 14 days after treatment. Cells lying between developing roots and cambium differentiate into vascular elements and establish vascular connections. *ca*, cambium; *wph*, wound phellogen.

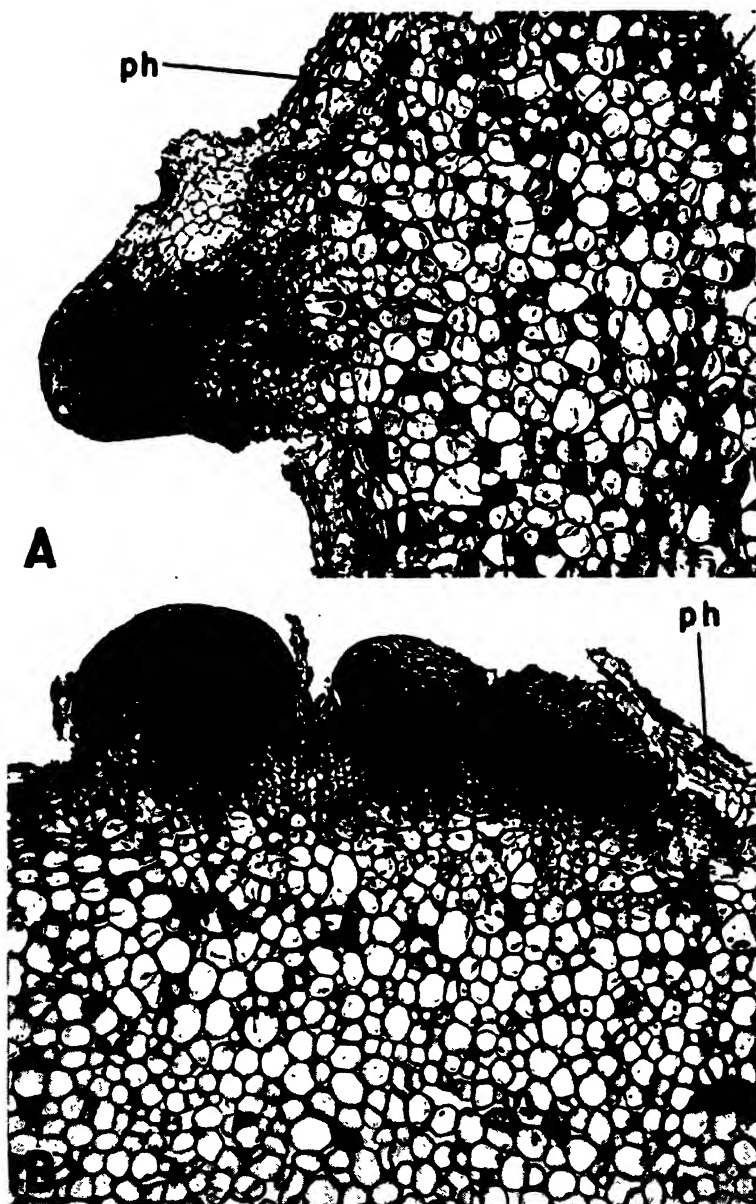


FIG. 13.—Longitudinal sections through outer portions of segment 14 days after peripheral treatment. Derivatives of phellogen, pericycle, and secondary phloem parenchyma form root primordia that rupture the periderm. Outer cells of developing roots dying; little cellular differentiation at base of root primordia (*cf.* figs. 11, 12). *ph*, phellogen.



FIG. 14.—Longitudinal sections through outer portions of segments 14 days after lateral treatment. *A*: intense activity of derivatives of thick walled parenchymatous cells within parent cell walls. *B*: differentiation of derivatives of thick walled parenchymatous cells at base of developing root into tracheids and cambial elements. Four tracheids within parent cell wall. *ph*, phellogen; *tr*, tracheid; *ca*, cambial cell.

parenchymatous cells just inside the periderm are usually thick walled. Storage cells containing myrosin are found in both the phloem and xylem regions.

Lateral roots are derived from the pericycle in the very young root before much cambial activity has occurred (figs. 2, 3). The endodermis is not involved in lateral root formation, but is crushed very early by the developing root. After a phellogen has appeared in the outer layers of the pericycle, additional lateral roots develop only in association with a root trace.

DEVELOPMENT OF ROOTS AND BUDS FROM LATERAL ROOT TRACES.—In the control segments, the origin of roots and buds is always in close association with a lateral root trace. The first discernible activity is seen in the cells closely associated with the phellogen and the cambium of the lateral root trace. The phellogen and its immature derivatives are involved as well as the derivatives of the cambium (fig. 6A). Soon the xylem and phloem parenchyma of the lateral root trace and associated parenchymatous cells of the main root begin active division and a mass of meristematic tissue is formed which, as a result of its growth and enlargement, ruptures the periderm (fig. 6B). If the lateral root trace is situated at the morphological lower end of the segment, organization into a root proceeds with typical histogen formation. This organization is usually complete before the periderm is ruptured. Differentiation of cells in the developing root proceeds back to the lateral root trace, so that the phloem and xylem of the new root and those of the lateral root trace are connected. On the other hand, if the lateral root trace is situated at the morphological upper end of the segment, a bud may be organized instead of a root. The first distinguishable region of the bud is a well defined dermatogen, which is followed by the formation of leaf primordia through differential activity of cells in the meristem (fig. 6C). Differentiation of vascular elements takes place between the developing bud and the root trace (fig. 6D). At the base of a bud, a region in close association with the epidermis of the bud and the phellogen of the segment often becomes active. This activity later involves cortical parenchyma at the base of the bud and a root primordium is organized. Differentiation of vascular elements in the cells between the root primordium and the bud takes place, thus establishing vascular connections between them.

Organization of both roots and buds usually begins about 5 or 6 days after the segment is treated with pure lanolin. Some meristematic areas appear sooner than others, however, and various stages of development may be found around any single root trace. Certain areas may be more or less meristematic in any intact fleshy root; this activity is dependent upon the past environmental conditions of the root.

When 2 per cent naphthylacetic acid in lanolin is applied to the upper cut surface of a segment, only roots are organized in the lateral root trace areas. With indoleacetic acid a few buds may be formed, but not nearly so many as in the controls. The same regions seem to be involved in bud and root formation, the only difference being in the subsequent organization of the mass of meristematic tissue (fig. 7). Organization of roots takes place about 5 or 6 days after the segment is treated, the time relations thus being about the same as in the control segments.

DEVELOPMENT OF CUT SURFACES.—During the first 2 days the cut surfaces of the control segments exhibit no apparent changes except for the deposition of material on the cell walls along the surface. Cell divisions first appear in the young secondary phloem, five or six cell layers in from the lower cut surface. Within 4 days a well defined wound phellogen has formed across the entire lower cut surface. In addition there is some callus formation, owing mainly to cell elongation, although some cell divisions may occur. Callus production ceases on about the eighth day. The phellogen becomes comparatively inactive about this time also, but the cells of the callus remain alive for some time after the death of cells beneath them (fig. 9A). After about 10 days the wound phellogen is complete across the upper part of the segment (fig. 8A). Necrotic areas are formed at both cut surfaces, extending deeply into the xylem and phloem, and these increase in extent until the wound periderm is formed. Since the formation of a phellogen near the upper cut surface does not take place so rapidly as it does near the lower cut surface, the necrotic areas are much more extensive near the upper than near the lower surface.

If the segment includes a lateral root trace near the upper surface, meristems may develop in the region where the cambium of the root trace comes in close proximity to the wound phellogen. These

meristems develop only in those regions where the root trace extends through the cambial region and the phloem. None has been observed in association with the portion of the trace which extends through the xylem. If the lateral root trace occurs near the lower cut surface, the meristems will give rise to roots, while if the trace occurs near the upper cut surface, the meristems may differentiate as buds.

After the application of 2 per cent naphthylacetic acid, the cells near the upper cut surface follow the same general pattern of response as was found in the control segments, except that the death of cells takes place much more slowly (fig. 8*B*). The treatment, however, induces a much greater response in the cells near the lower surface. The wound phellogen, instead of becoming comparatively inactive after 8 days, proceeds to divide very actively, and adjacent cells also become active. Meristems, that later give rise to roots, originate from certain of these active regions. During the first 8 days the activity of the phellogen is confined chiefly to the phloem region (fig. 9*B*). By the tenth day, however, areas of wound phellogen formation may be found in the xylem. This is in contrast to the control, in which the phellogen extended across the surface at the end of 8 days. The xylem activity varies considerably. In some segments a well developed phellogen may develop across the entire xylem area, while in others it may be localized.

Those roots which develop from meristematic areas not associated with a lateral root trace are derived from phloem parenchyma, xylem parenchyma, and cambial regions. Even myrosin storage cells may be involved (fig. 10*B*). Roots arise most frequently in the phloem and cambial regions. Root organization from these meristems is first observed about 12 days after treatment. After the development of histogens in the root, differentiation of vascular elements takes place between the root and the main cambial region (figs. 11, 12). Differentiation of scattered phloem and xylem elements and scattered secondary cambiums may take place in active regions not in association with a developing root (fig. 10*A*). The roots from the xylem parenchyma usually originate near a strand of tertiary phloem.

Treatment of the cut surfaces also induced the differentiation

of more vessels, sieve tubes, and companion cells from the derivatives of the main cambium, with consequent reduction in the relative number of new phloem and xylem parenchyma cells.

In both control and treated segments, a vessel that abuts the cut surface may be crossed by the wound phellogen. The dividing cells on either side of the vessel force their way into its lumen through pits. There they divide and enlarge until the phellogen is complete across the vessel. Divisions may proceed until the lumen is partially filled with these cells and has the appearance of a vessel with tyloses.

RESPONSE TO LATERAL TREATMENT.—The first noticeable activity as a result of lateral application of indoleacetic or naphthylacetic acids is in the phellogen and the adjacent five or six parenchymatous layers. Later these inner cells become more active, and they may either form localized phellogens, resulting in the death of cells exterior to them, or continue to divide without forming a phellogen (fig. 144). In the latter case organization of the cells into roots may occur (fig. 13). Organization may take place in the outermost parenchymatous layers, or it may take place ten or fifteen cell layers in from the phellogen, and orientation of the new root axis may be in any direction. These roots do not develop further but soon die. A few tracheids and cambial elements may be differentiated at the base of the partially developed roots.

Discussion

The horseradish root has frequently been used for studying regenerative phenomena in plants. Years ago RECHINGER (21) described the gross development of roots and buds from the regions of the root traces. He found that transverse sections of the root only 1.5 mm. thick were capable of producing buds. Later KUPFER (14) repeated this work and in some instances claimed to have obtained buds from the cut surfaces. It seems likely that these buds developed from root traces near the cut surface, for PRIESTLEY and SWINGLE (20) could not confirm KUPFER's work, nor could the present writer. If segments of the horseradish root are cut near a lateral root trace, buds will develop in association with the root trace near the upper surface and break through, thus giving the appearance that they actually arise from the surface.

BEALS (2) did a limited amount of histological work with the horseradish root and stated that buds developed from cambial cells. This was not found to be strictly the case in the material presented here, although it is true that cambial derivatives may be involved in root and bud production. The development of roots from the cut surfaces is similar to that described by PRIESTLEY and SWINGLE (20) for untreated segments of sea kale root.

It appears that the application of either indoleacetic or naphthylacetic acid, in the relatively high concentrations used here, inhibits the organization and development of buds but stimulates the organization and development of roots. Similar results have recently been reported for sea kale roots (23). This is in agreement with most recorded work (4, 25). GREENLEAF (8), however, working with *Nicotiana*, found stimulation of shoot production from the cut surfaces of decapitated stems when they were treated with 1 per cent indoleacetic acid in lanolin. He found it necessary to remove all lateral buds in order to obtain adventitious buds from the callus. GOLDBERG'S (7) studies show that the application of indoleacetic acid to the stems of decapitated cabbage seedlings stimulates the production of roots and buds from the callus. She did not remove the lateral buds. Decapitated plants, when left untreated, produced comparatively few buds from the callus. BEAL (1) reports the development of buds in the leaf axis of *Lilium harrisii* when the stem is decapitated and the cut surface is treated with 3 per cent indoleacetic acid in lanolin. Untreated plants do not produce axillary buds. In this case there is no callus formed in the region of bud development. With the same treatment two other species of lily formed roots but not buds. LINK and EGGERS (16) found that the application of relatively high concentrations of indoleacetic acid to the decapitated hypocotyls of flax plants inhibited the formation of hypocotyledonary buds. They further report (17) that in certain low concentrations there is a stimulation of bud development. The interpretation of these varying results remains for the future.

When growth substances were applied to either the morphological upper or lower surfaces of the horseradish root segments, roots developed from the lateral surfaces and from cells adjacent to the lower cut surface, but never from the upper cut surface. Yet

when the growth substances were applied peripherally, roots developed from the outer parenchymatous cells in the region of application near the upper surface. Other workers have shown that when the stems of various plants are treated with relatively high concentrations of auxin-like compounds, numerous adventitious roots are produced near the treated regions (1, 3, 6, 7, 9, 10, 11, 13, 15, 22). When the whole fleshy axis of the horseradish plant was cut longitudinally and the cut surfaces treated, the development of roots from the lateral root traces was confined to two areas: one composed of the stem proper and the root-stem junction, and the other at the extreme lower end of the root. In the cases where the whole fleshy axis was cut transversely into 4 cm. segments, the extreme lower end responded the same as did the majority of the root segments, while the segment that contained the stem region was by far the most active in both root and shoot production. Although it is possible that the activity in the upper part of the fleshy structure was related mainly to the much greater number of traces coming to the surface there, many other factors undoubtedly also played a role.

The response of the root was much more intense when the growth substances were applied to the upper cut surfaces of the segments than when they were applied to the lower cut surfaces. This would indicate that the substances travel downward through the segment much more readily than they do upward. These observations are in accord with the work of previous investigators (4, 25). If this is true, the comparatively small amount of meristematic activity in the xylem of the lower portions of the segments might be explained on the basis of the accumulation of the growth substances (and any other compounds through which they might act) in the phloem and cambial regions, thus making the xylem comparatively low in these substances. This is further substantiated by the fact that cellular activity was most intense in the regions of the xylem where tertiary strands of phloem were developed.

Either the main phellogen of the root or a wound phellogen was involved in all cases of root and shoot production in the mature root. Formative activity was usually greatest in the region of the cambium of the lateral root trace. Meristems, resulting in the production of roots from phloem and xylem parenchyma, were formed only after

wound phellogens had been produced. Derivatives of these phellogens made up the outer portions of the developing roots. In the control segments, roots and buds were formed readily from the regions where the lateral root traces approached a phellogen, but without root traces there was no formation of buds and only an occasional root from the cut surfaces.

Naphthylacetic acid was much more effective than indoleacetic acid in producing response in the horseradish. Its superiority may be due to its greater stability or to its greater mobility (or both) within the plant. Peripheral application of naphthylacetic acid is more effective than application at the lower cut surface. Indoleacetic acid, when applied to the bottom of the segment, is more effective than when applied peripherally. Distribution of these compounds from a lateral application would necessitate their movement through the phellem to the phellogen, and then either down the phellogen or outer parenchymatous cells, or through a great mass of phloem parenchyma cells to the sieve tubes. Apparently naphthylacetic acid is more mobile through these tissues than is indoleacetic acid.

When the segments were washed with water for 24 hours before being treated, no callus was formed. This would tend to indicate either the loss of some building materials such as amino acids and sugars, a more specific substance like tyrosine (18), or even more intricate substances that control the formation of callus similar to (or identical with) the "wound hormones" reported by several workers (5, 24). These results suggest that the application of indoleacetic and naphthylacetic acids did not cause callus formation directly, but merely affected the distribution of some controlling substances. The greatly increased susceptibility of the washed segments to invasion by micro-organisms may indicate that some substance or substances which give some degree of immunity were leached out. There remains, of course, the possibility that the segments were more susceptible to invasion during the 24 hours they were in the water.

In both the control and treated segments, cellular differentiation into vascular elements (in the region of lateral root traces) took place only after roots and buds were well formed. Then vascular connec-

tions between the developing root or shoot and the lateral root trace were differentiated. When roots developed from the lower cut surface of treated segments, differentiation proceeded between the developing root and the cambium. However, certain active areas not involved in root production differentiated small scattered strands of phloem and xylem elements and secondary cambiums. Peripheral application of the growth substances produced meristematic areas so far away from the cambium that cellular differentiation into vascular elements occurred in localized areas at the base of a developing root, but further centripetal differentiation did not occur. These roots soon perished, probably from lack of vascular connections.

Summary

1. In the young horseradish root, lateral roots develop from the pericycle or its derivatives, resulting in crushing of the endodermis.
2. In the mature root, or transverse segments of it, buds originate in the region where the cambium of the upper root traces comes in contact with the phellogen. Roots originate from lower root traces in a similar manner. Derivatives of the cambium, phellogen, pericyclic parenchyma, phloem parenchyma, and xylem parenchyma may all be involved in the development of buds and roots. Occasionally a root may be formed from the lower cut surface without a root trace being involved.
3. Application of relatively high concentrations of indoleacetic and naphthylacetic acids inhibits shoot production and stimulates root production. Under the influence of these compounds, roots are organized in the regions where buds would otherwise have organized. In addition, the morphologically lower cut surfaces of the treated segments produce numerous roots from derivatives of the wound phellogen, phloem parenchyma, xylem parenchyma, and cambium. There is no root organization from the upper cut surface unless a lateral root trace is involved.
4. Peripheral application of indoleacetic or naphthylacetic acid induces activity in the outer parenchymatous cells of the root, which culminates in the formation of new roots that soon perish.
5. Placing the cut segments in running water for 24 hours before

treatment inhibits the production of callus and results in an increased invasion by micro-organisms.

6. Various extracts of the horseradish plants were ineffective in the stimulation of the production of buds.

The writer is indebted to members of the botany department of the University of Chicago for helpful suggestions made during the course of this study.

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CYTOLOGICAL STUDIES IN RELATION TO
THE CLASSIFICATION OF THE
GENUS *CALOCHORTUS*¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 498

J. M. BEAL

(WITH FORTY FIGURES)

Introduction

The species of *Calochortus* are common and widespread liliaceous plants of the Pacific Coast. They range from Canada to Guatemala and eastward to Nebraska and the Dakotas. Within these limits no considerable region is without one or more species. The genus is found naturally only in western North America, with a greater number of species present in California than in any other similar area.

Not only is the range of the genus extensive but that of several of the species is great, and the conditions of soil and climate under which they grow show great variation. Under such diverse environmental conditions it is perhaps natural to expect considerable variation in many of the species, and this has been repeatedly observed by those who have collected the plants in their natural habitats. Variation has been equally apparent under garden conditions. According to PURDY (private correspondence with the writer), hybridization appears to be of infrequent occurrence. In general, the various species produce seeds in abundance. The genus has proved to be a difficult one for systematists, and there is still question concerning the determination and taxonomic arrangement of many of the species.

The present investigation shows that the number and morphology of the chromosomes are characteristics which aid in determining the natural relationships among the species. No attempt will be made to review all the taxonomic literature pertaining to the genus, but

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three treatments which have some bearing upon the cytological findings will be briefly mentioned.

In 1879, WATSON (7) divided the genus into three sections, *Eucalochortus*, *Mariposa*, and *Cyclobothra*, placing eleven species under the first section, fourteen under the second, and six under the third. The third section comprises the Mexican species, while the first and second include those found north of Mexico. One species, *C. catalinae*, was unassigned because the flowers were unknown to him.

The second is that of PURDY (6), published in 1901. Only species occurring north of Mexico are considered in his monograph. These are divided into the *Eucalochortus* and *Mariposa* sections, essentially as was done by WATSON. PURDY includes sixteen species under the first section and twenty-four under the second, making a total of forty species as compared with WATSON'S twenty-five. The group arrangement under the sections differs somewhat in the two treatments, but their criteria for sectional distinction are essentially the same.

The third treatise is that of ABRAMS (1), published in 1923. He makes no formal separation of the genus into sections. The key to the species is arranged in two main divisions: capsule winged on the angles and capsule not winged. All the species examined by the writer which possess 20 (or 40) somatic chromosomes fall into the first category, while those which show some other number fall into the second. The capsule may perhaps furnish the most significant morphological structures in the genus.

Although Index Kewensis lists seventy-nine species for the genus, it is generally agreed among those American taxonomists who have worked most carefully with the group that there are approximately fifty valid species.

NEWTON (5) has reported the number and discussed briefly the chromosome morphology in the following ten species:

EUCALOCHORTUS	2n	n
<i>C. albus</i> Dougl.....	20	10
<i>C. amabilis</i> Purdy.....	20	10
<i>C. benthami</i> Baker.....	20	10
<i>C. marweanus</i> Leicht.....	20	

MARIPOSA

<i>C. plummerae</i> Greene.....	18	
<i>C. clavatus</i> S. Wats.....	16	
<i>C. catalinae</i> S. Wats.....	14	7
<i>C. luteus</i> Douglas.....	14	
<i>C. venustus</i> var. <i>eldorado</i> Purdy....	14	7
<i>C. vesta</i> Purdy.....	28	14

All these species except *C. benthami* have been examined by me and the numbers reported by NEWTON confirmed. All the available material of *C. luteus* has shown a somatic number of 20 or 21 chromosomes, indicating that it was triploid, especially as two varieties of the species, *C. luteus* var. *citrinus* and *C. luteus* var. *oculatus*, both possess a diploid number of 14 chromosomes. NEWTON observed the striking similarity of size and form variations among the chromosomes of the species assigned to certain groups, as well as the occurrence of certain forms in all the species. This condition has been observed during the present study.

Material and methods

Although the material has been obtained from several sources, bulbs of most of the species and varieties investigated were secured from MR. CARL PURDY, Ukiah, California, whose determinations in the main have been accepted by the writer. *C. gunnisoni* and *C. nuttallii* were secured from MR. CLAUDE A. BARR, Smithwick, South Dakota. MR. MARION OWNBEY, Missouri Botanical Garden, kindly sent identified material of *C. luteus*, *C. elegans*, and *C. selwayensis*, and MISS VADA H. ALLEN, Department of Botany, University of Idaho, supplied bulbs of *C. elegans*. *C. aureus* was also obtained from MR. RALPH O. BAIRD, Gallup, New Mexico. To all these collaborators the writer expresses his gratitude.

The bulbs were potted during the autumn in a sandy loam soil and placed in a coldframe until freezing temperatures occurred, when they were placed in one of the cooler rooms of the greenhouses. As roots began to emerge their tips were cut off, and in most instances, were fixed in both LaCour's 2 BE and Navashin's solutions. On the whole, LaCour's fixative gave the better results, and most of the figures are from slides fixed in this solution. After fixation, the ma-

terial was run up through the usual schedule and imbedded in paraffin. Transverse sections were cut at 20-25 μ in thickness and stained according to the gentian violet-iodine method.

A number of the species produced flower buds from which anthers were taken for the study of meiosis. To determine the stage in meiosis, one anther from each bud examined was crushed in acetocarmine. When appropriate stages were found, the remaining anthers were immediately smeared on a clean slide, fixed for two hours in Navashin's solution, and stained with the gentian violet-iodine stain. For comparison with the somatic chromosomes at comparable stages, only figures of the first meiotic metaphases during microsporogenesis are shown.

All drawings were made at table level with the aid of a camera lucida, using a 15 \times compensating ocular and a 90 \times apochromatic objective, n.a. 1.40, with a yellow-green filter. The magnification is approximately 2450.

Observations and taxonomic arrangement

The arrangement of the sections and subsections presented in the following classification is that supplied by MR. MARION OWNBEY, who is engaged in a monographic study of the genus at the Missouri Botanical Garden. The chromosome numbers of all the species and varieties here included were sent to him during the autumn of 1938. For some of these species he had determined the numbers, and in addition had made counts on some not listed in this paper. In all cases our determinations have been in agreement and the classification herewith presented appears to represent a natural arrangement based upon both cytological and morphological relationships. It is a pleasure to acknowledge my indebtedness to MR. OWNBEY for his kindness in furnishing the classification and permitting it to be used here before he publishes it in its final and more complete form.

SECTION I. EUCALOCHORTUS

Under section I are included all species and varieties which possess 10 as the basic number of chromosomes and which have capsules winged on the angles. This necessitates the transfer to section I of those species which PURDY (6) places under section II as the group

Oregon Mariposas. Four of the five species listed by PURDY for the group possess 10 as the basic number of chromosomes. A fifth species, *C. lyallii*, mentioned by PURDY as a synonym for *C. elegans* var. *nanus* (which it is not), has a like number. These species also have ovate, three-winged capsules, and therefore are placed under subsection I D, Nitidi, forming a natural and coherent group on both cytological and morphological grounds.

Subsection I A. Eleganti

C. tolmiei Hooker & Arnott. —According to OWNBEY, *C. tolmiei* should include *C. maweanus* Leicht. and its varieties. Three varieties of *C. maweanus* were secured from PURDY, var. *major* Purdy, var. *purpurascens* Purdy ined., and var. *roseus* Purdy. All possess the same number of chromosomes, and their karyotypes are so similar as to be indistinguishable. For this reason only one of them, var. *major*, is illustrated, and this has a diploid number of 20 chromosomes (fig. 1) with 10 pairs at metaphase I of meiosis (fig. 1a).

C. elegans Pursh. —Diploid, 20 chromosomes (fig. 2). Karyotype similar to preceding.

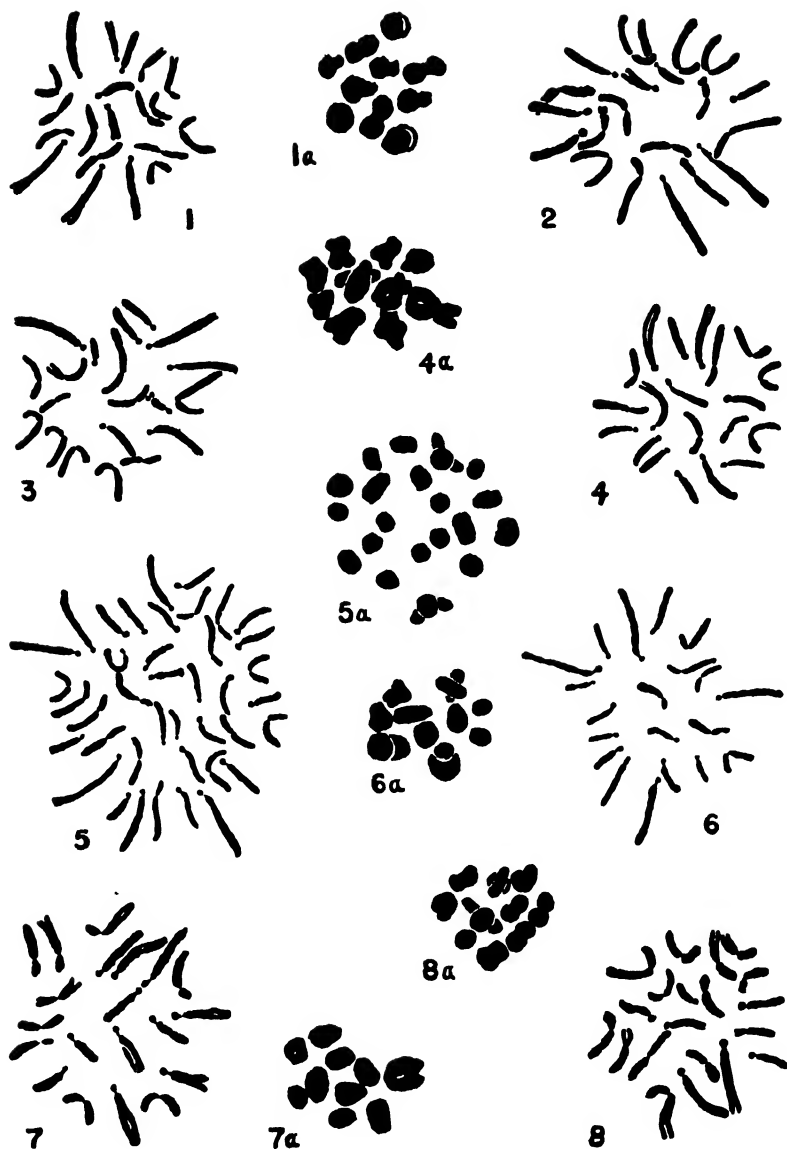
C. selwayensis St. John. —Diploid, 20 chromosomes (fig. 3). Karyotype scarcely distinguishable from that of *C. elegans*, of which it may be merely a variety.

C. apiculatus Baker. —Diploid, 20 chromosomes (fig. 4); 10 pairs at metaphase I of meiosis (fig. 4a). The karyotype of this species differs slightly from those of the preceding three. The differences may perhaps result from a certain amount of translocation or inversion, or a combination of both processes.

Subsection I B. Nudi

C. uniflorus Hooker & Arnott (*C. lilacinus* Kellogg). —Diploid, 40 chromosomes (fig. 5); 20 pairs at metaphase I of meiosis (fig. 5a). The various forms of chromosomes occur in quadruplicate and hence this species is a tetraploid. Pairing appears to be regular in meiosis with no evidences of multivalent association. There is some indication of secondary association in figure 5a however.

C. nudus S. Wats. —Diploid, 20 chromosomes (fig. 6). Karyotype strikingly similar to that of the preceding species, with the various forms of chromosomes present in both.



FIGS. 1-8.—Fig. 1, *C. tolmiei*, root tip metaphase. Fig. 1a, same, metaphase I. Fig. 2, *C. elegans*, root tip metaphase. Fig. 3, *C. selwayensis*, root tip metaphase. Fig. 4, *C. apiculatus*, root tip metaphase. Fig. 4a, same, metaphase I. Fig. 5, *C. uniflorus*, root tip metaphase. Fig. 5a, same, metaphase I. Fig. 6, *C. nudus*, root tip metaphase. Fig. 6a, same, metaphase I. Fig. 7, *C. amabilis*, root tip metaphase. Fig. 7a, same, metaphase I. Fig. 8, *C. amoenus*, root tip metaphase. Fig. 8a, same, metaphase I.

Subsection I C. Pulchelli

C. amabilis Purdy.—Diploid, 20 chromosomes (fig. 7); 10 pairs at metaphase I of meiosis (fig. 7a).

C. amoenus Greene.—Diploid, 20 chromosomes (fig. 8); 10 pairs at metaphase I of meiosis (fig. 8a).

C. albus Douglas.—Diploid, 20 chromosomes (fig. 9); 10 pairs at metaphase I of meiosis (fig. 9a).

The karyotypes of the three species embraced under this subsection are so similar that it would be practically impossible to distinguish them from one another, except for the fact that *C. albus* has one pair of chromosomes with attachment constrictions so elongated as to simulate, if not actually to constitute, a satellited condition.

Subsection I D. Nitidi

C. lyallii Baker.—Diploid, 20 chromosomes (fig. 10); 10 pairs at metaphase I of meiosis (fig. 10a).

C. howellii S. Wats.—Diploid, 20 chromosomes (fig. 11); 10 pairs at metaphase I of meiosis (fig. 11a).

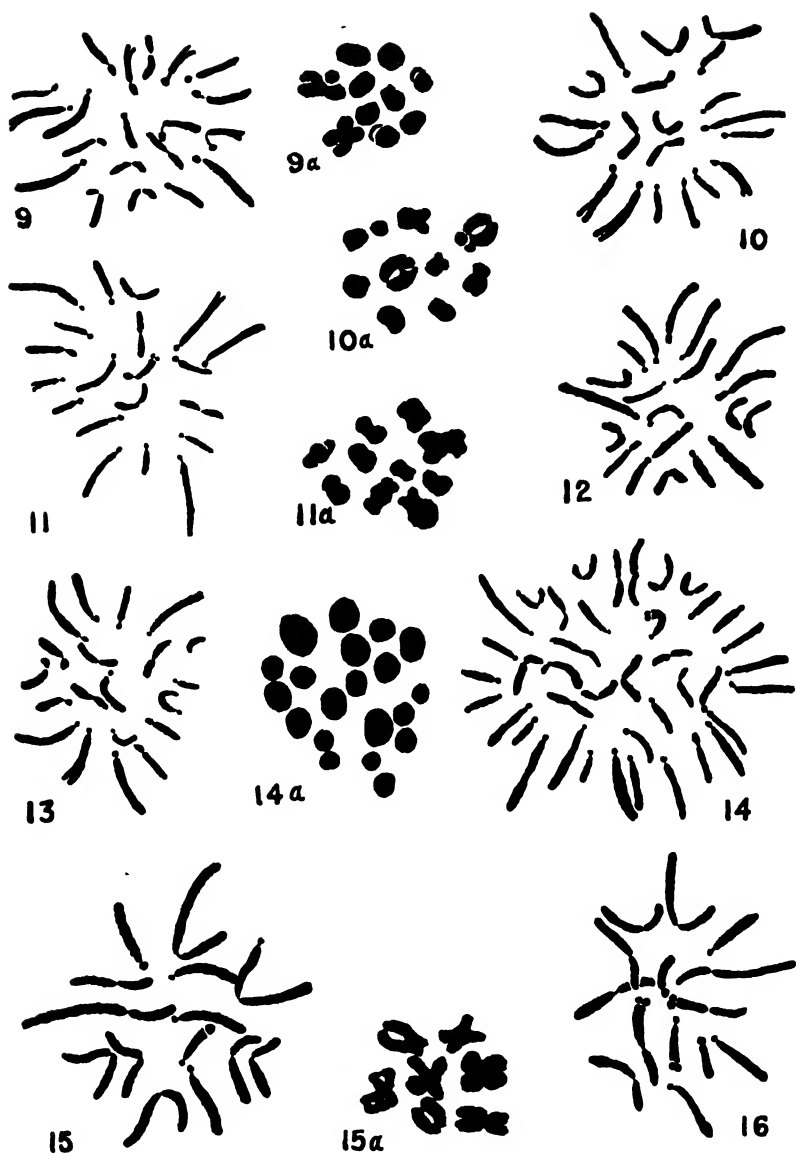
C. nitidus Douglas (*C. eurycarpus* S. Wats.).—Diploid, 20 chromosomes (fig. 12).

C. greenii S. Wats.—Diploid, 20 chromosomes (fig. 13).

C. pavonaceus Fernald (*C. nitidus* of several authors, not of Douglas).—Diploid, 40 chromosomes (fig. 14); 20 pairs at metaphase I of meiosis (fig. 14a). As in *C. uniflorus*, the various types of chromosomes occur in quadruplicate and this is also a tetraploid. PURDY placed this species, together with *C. nitidus* (*C. eurycarpus*), *C. greenii*, and *C. howellii*, under the section Mariposa and the group Oregon Mariposas, and stated it was "the largest flowered of the group." Because of the chromosome numbers and the winged capsules it is evident that all these species belong under the section Eucalochortus.

SECTION II. MARIPOSA

This section comprises those species with a basic number of chromosomes other than 10, and which have capsules not winged. The section is decidedly more diverse in the number of chromosomes and karyotypes than is section I. Somatic numbers of 12, 14, 16,



FIGS. 9-16.—Fig. 9, *C. albus*, root tip metaphase. Fig. 9a, same, metaphase I. Fig. 10, *C. lyallii*, root tip metaphase. Fig. 10a, same, metaphase I. Fig. 11, *C. howellii*, root tip metaphase. Fig. 11a, same, metaphase I. Fig. 12, *C. nitidus*, root tip metaphase. Fig. 13, *C. greenii*, root tip metaphase. Fig. 14, *C. pavonaceus*, root tip metaphase. Fig. 14a, same, metaphase I. Fig. 15, *C. catalinae*, root tip metaphase. Fig. 15a, same, metaphase I. Fig. 16, *C. splendens*, root tip metaphase.

18, 20, 21, 28, and 32 chromosomes have been observed, the numbers 28 and 32 being from tetraploids and 20 and 21 from triploids.

Subsection II A. Venusti

C. catalinae S. Wats.—Diploid, 14 chromosomes (fig. 15); 7 pairs at metaphase I of meiosis (fig. 15a). One or both of the long medianly constricted chromosomes may possess satellites.

C. splendens Douglas.—Diploid, 14 chromosomes (fig. 16).

C. splendens var. *rubra* Purdy.—Diploid, 14 chromosomes (fig. 17); 7 pairs at metaphase I of meiosis (fig. 17a). Karyotype scarcely distinguishable from the species.

C. leichtlinii Hooker.—Diploid, 14 chromosomes (fig. 18). Chromosomes somewhat shorter than in preceding species but otherwise similar.

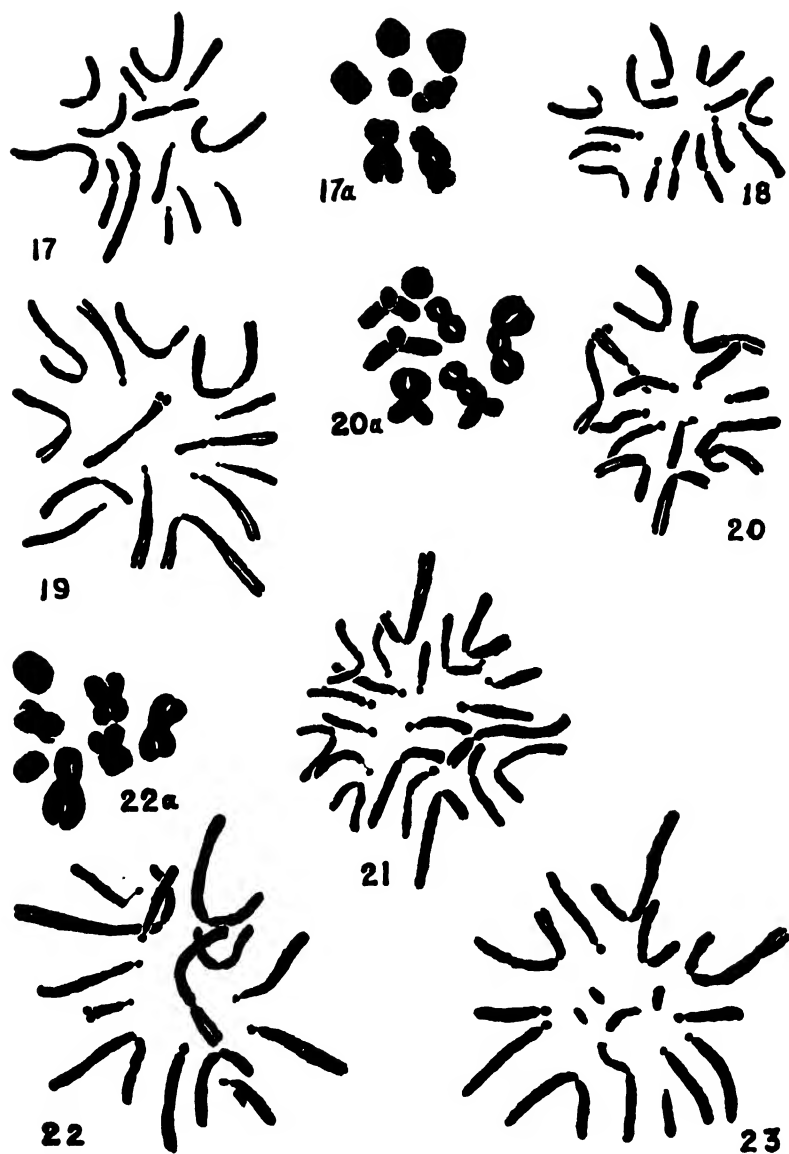
C. venustus Douglas.—Diploid, 14 chromosomes (fig. 19). This and the succeeding species of the subsection have somewhat longer and larger chromosomes than any of the other species examined.

C. venustus var. *caroli* Cockerell.—Diploid, 14 chromosomes (fig. 20); 7 pairs at metaphase I of meiosis (fig. 20a). One bulb was triploid, showing 21 chromosomes in its root tip cells (fig. 21). Karyotypes similar to that of the species.

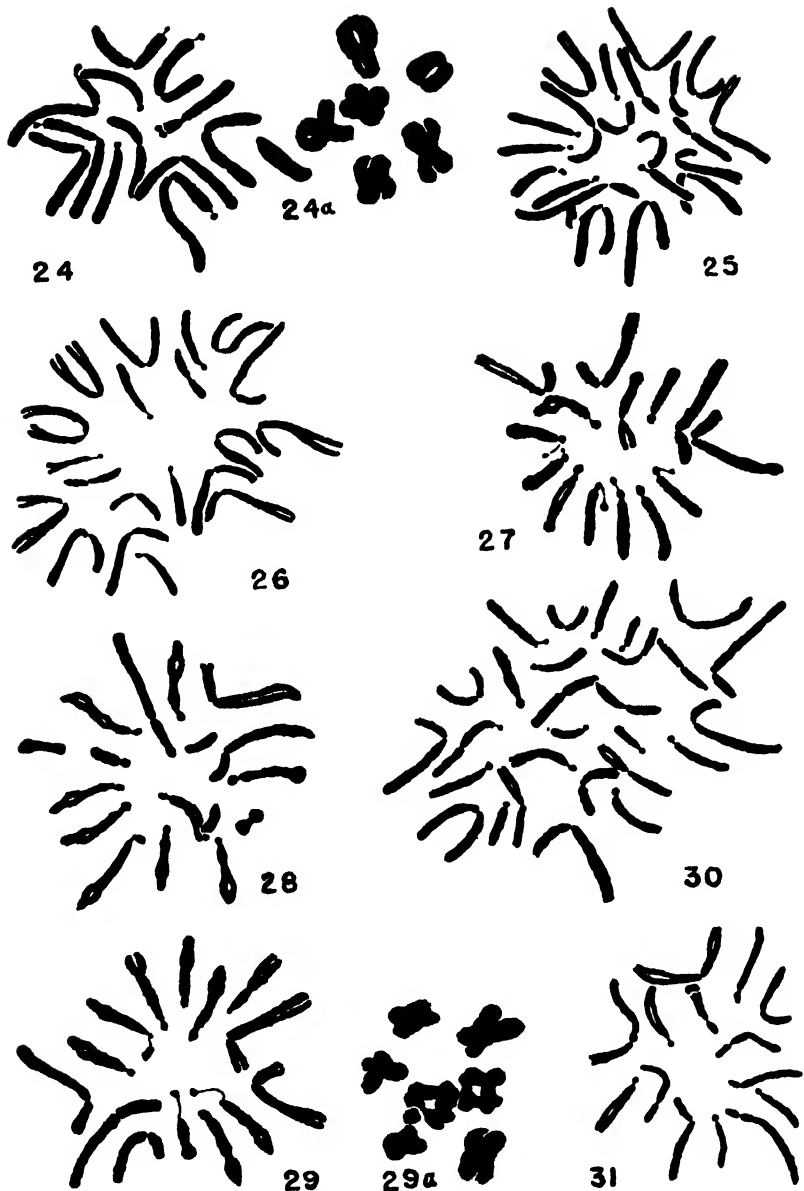
C. venustus var. *purpurascens* S. Wats.—Diploid, 14 chromosomes (fig. 22); 7 pairs at metaphase I of meiosis (fig. 22a). Fragmentation appears to be of frequent occurrence, as a number of the preparations showed a condition similar to that of figure 23. But neither the bulbs nor the plants produced from them showed external evidences of abnormalities.

C. superbus Purdy.—Diploid, 12 chromosomes (fig. 24); 6 pairs at metaphase I of meiosis (fig. 24a). HOWELL (2) states that "*C. superbus* seems most closely related to *C. luteus*." It seems probable that *C. superbus* may have been derived from *C. luteus* through fusion and translocation between two of its chromosomes, designated as chromosomes 2 and 6 (figs. 39, 40), with elimination of the attachment constriction of chromosome 2. This possibility is discussed in greater detail in a later section.

C. luteus Douglas.—NEWTON reported the diploid number for this species as 14, but all material available to me from both PURDY and



FIGS. 17-23.—Fig. 17, *C. splendens* var. *rubra*, root tip metaphase. Fig. 17a, same, metaphase I. Fig. 18, *C. leichlinii*, root tip metaphase. Fig. 19, *C. venustus*, root tip metaphase. Fig. 20, *C. venustus* var. *caroli*, root tip metaphase. Fig. 20a, same, metaphase I. Fig. 21, same, root tip metaphase from triploid bulb. Fig. 22, *C. venustus* var. *purpurascens*, root tip metaphase, one fragment present. Fig. 23, same, root tip metaphase, several fragments present.



FIGS. 24-31.—Fig. 24, *C. superbus*, root tip metaphase. Fig. 24a, same, metaphase I. Fig. 25, *C. luteus*, root tip metaphase, 21 chromosomes. Fig. 26, same, root tip metaphase, 20 chromosomes. Fig. 27, *C. luteus* var. *citrinus*, root tip metaphase. Fig. 28, same, root tip metaphase showing fragments. Fig. 29, *C. luteus* var. *oculatus*, root tip metaphase. Fig. 29a, same, metaphase I. Fig. 30, *C. vesta*, root tip metaphase. Fig. 31, *C. macrocarpus*, root tip metaphase.

OWNBEY has shown either 20 (fig. 25) or 21 (fig. 26) chromosomes in root tip cells, with the latter number occurring far more commonly. The various chromosome forms occur in triplicate, and there is therefore every reason to consider this material to be triploid. No meiotic material has been available and it has not been possible to observe the behavior of the chromosomes during the meiotic pro-phases. OWNBEY collected apparently normal seeds produced from some of the bulbs examined, and apparently seed production is common in this triploid form.

C. luteus var. *citrinus* (Baker) S. Wats.—Diploid, 14 chromosomes (fig. 27). Fragmentation appears to be common in this variety also (fig. 28). Five chromosomes in figure 27 show satellites, possibly as the result of translocated fragments.

C. luteus var. *oculatus* S. Wats.—Diploid, 14 chromosomes (fig. 29); 7 pairs at metaphase I of meiosis (fig. 29a). Two chromosomes in figure 29 show satellites and a third an attached fragment.

C. vesta Purdy.—Diploid, 28 chromosomes (fig. 30). This tetraploid form is placed as a variation or color form of *C. venustus* by some taxonomists, but it appears to merit specific rank on both cytological and morphological grounds. Its chromosomes resemble those of *C. luteus* more closely than those of *C. venustus*, and apparently it is more closely related to the former than to the latter.

Subsection II B. Macrocarpi

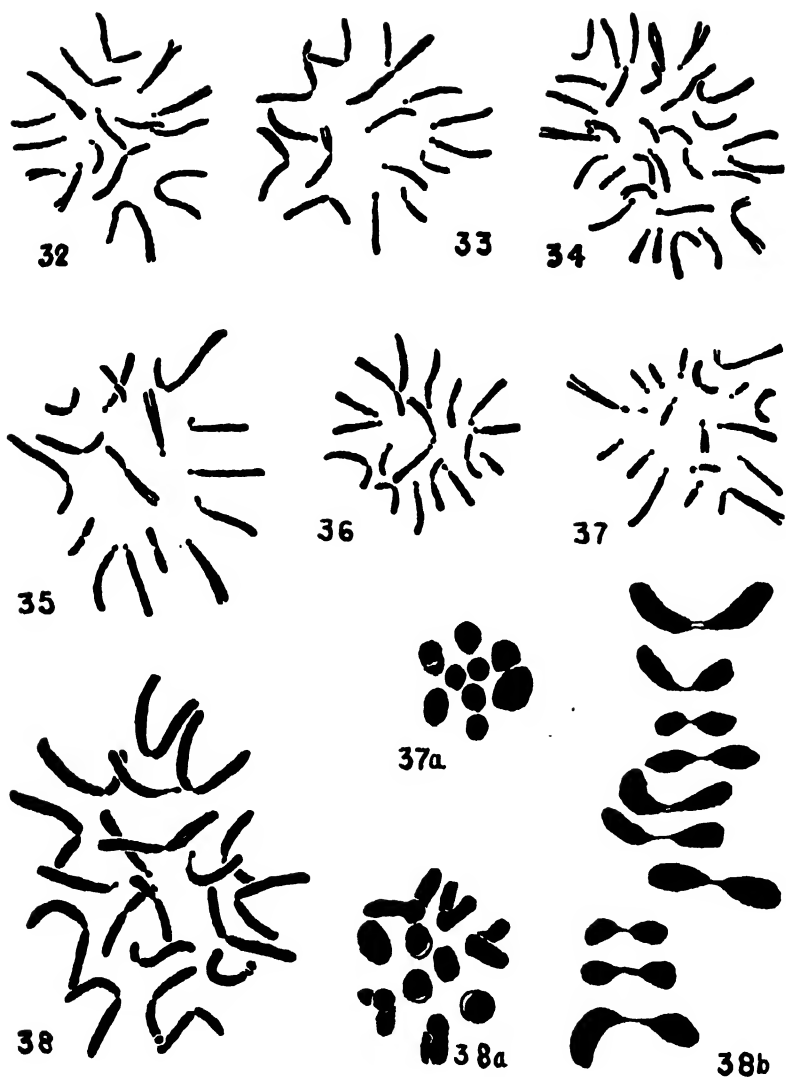
C. macrocarpus Douglas.—Diploid, 14 chromosomes (fig. 31). This species appears to combine some characters of the preceding subsection with some of the following subsection, but differs from either in certain other respects. And while it is probably more closely related to subsection II C on the whole, it possesses 14 rather than 16 as the diploid complement of chromosomes and appears to justify assignment to a distinct subsection.

Subsection II C. Nuttaliani

C. clavatus S. Wats.—Diploid, 16 chromosomes (fig. 32).

C. kennedyi Porter.—Diploid, 16 chromosomes (fig. 33).

C. aureus S. Wats.—Diploid, 32 chromosomes (fig. 34). Again the various sizes of chromosomes occur in quadruplicate, and this species is tetraploid.



FIGS. 32-38.—Fig. 32, *C. clavatus*, root tip metaphase. Fig. 33, *C. kennedyi*, root tip metaphase. Fig. 34, *C. aureus*, root tip metaphase. Fig. 35, *C. nuttallii*, root tip metaphase. Fig. 36, *C. gunnisoni*, root tip metaphase. Fig. 37, *C. plummerae*, root tip metaphase. Fig. 37a, same, metaphase I. Fig. 38, *C. luteus* var. *citrinus* "vivid," root tip metaphase, 20 chromosomes. Fig. 38a, same, metaphase I. Fig. 38b, same, bivalents drawn separately to show complete terminalization of chiasmata at metaphase I.

C. nuttallii Torrey.—Diploid, 16 chromosomes (fig. 35). These four species form a coherent group with their markedly similar karyotypes and similar morphological characters.

Subsection II D. Gunnisoniani

C. gunnisoni S. Wats.—Diploid, 18 chromosomes (fig. 36). This species also requires a subsection of its own because of its cytological and morphological differences from the members of the preceding subsections.

SECTION III. CYCLOBOTHRA

Under section III are included those species with linear unwinged fruits and thick fibrous-reticulate bulb coats.

C. plummerae Greene.—Diploid, 18 chromosomes (fig. 37); 9 pairs at metaphase I of meiosis (fig. 37a). Its chromosomes are somewhat thinner than those of any of the other species examined and the karyotype differs in some other respects. It should be interesting to examine other species of the Cyclobothra section for comparison with *C. plummerae* as well as with species of the other two sections. Would they show a similar variation in chromosome numbers and morphology such as is shown in the Mariposa section, or would they possibly have a single basic number throughout, as occurs in the Eucalochortus section?

DOUBTFUL FORM

A variety secured from PURDY under the name of *C. luteus* var. *citrinus* "vivid" showed a diploid number of 20 chromosomes in root tip cells (fig. 38) and 10 pairs at metaphase I of meiosis (fig. 38a). There is complete terminalization of chiasmata as shown in figure 38b. In this figure the chromosomes, drawn separately, are from a single sporocyte. A similar condition was observed in a number of the sporocytes. This is probably an autotriploid among whose homologous chromosomes translocations and inversions have occurred so as to change their homologies and pairing relations.

FRAGMENTATION

Fragments have been observed in a few of the species and varieties investigated, notably in *C. luteus* var. *citrinus* (fig. 28) and *C. venustus* var. *purpurascens* (fig. 23). A majority of the root tips from

these two varieties have shown them, and they have been seen in the sporocytes also. In some cases a fragment becomes attached laterally to a chromosome (fig. 22), in others terminally to produce a satellited condition (fig. 29). In most instances they remain apparently unattached, although their behavior has not been followed in detail. But neither bulbs, roots, nor plants possessing fragments appear different in any recognizable way from those not possessing them.

Discussion

The *Eucalochortus* section is much more uniform in practically all respects than is the *Mariposa* section. All the species examined under the former section show a basic number of 10 chromosomes and relatively little variation in karyotypes, while in the latter section there are species with basic numbers of 6, 7, 8, and 9 chromosomes. Seven is suggested as the original basic number for the *Mariposa* section and perhaps also for the genus. Species which possess some other number have possibly been derived from one or more of the species in which 14 is the somatic number of chromosomes by doubling the number in one or both types of spores (and gametes) to produce the triploid and tetraploid forms; or as a result of segmentation or translocation, with or without inversions; or through a combination of these processes.

For example, *C. superbus*, with $2n = 12$ chromosomes, may have been derived from *C. luteus*, $2n = 14$ chromosomes, through fusion and translocation between two chromosomes of the latter, one of which, designated as 2 (fig. 40), has a subterminal attachment constriction, and the other, designated as 6, has a submedian one. If during the prophases of a nuclear division in the early development of an embryo, chromosome 2 should become attached just below its attachment constriction to the shorter arm of chromosome 6, at or near its distal end, followed by breakage and detachment of the centromere (kinetic body) with the accompanying small knob of chromosome 2, a new chromosome carrying essentially all the genes of both former chromosomes would result. Since the detached fragment carries few, possibly no, genes, it would probably be eliminated during the division which follows. As a consequence two cells would

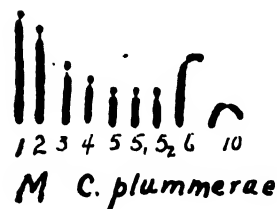
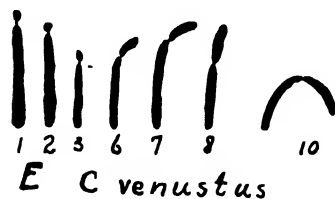
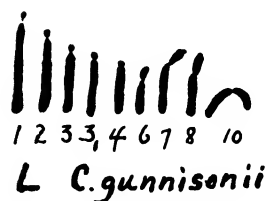
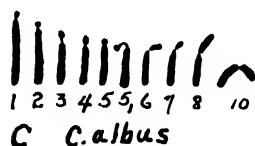
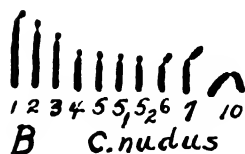
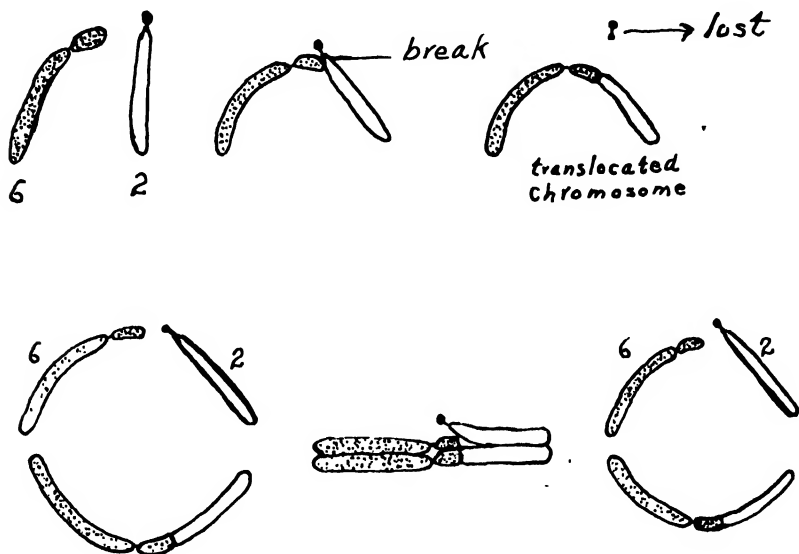


FIG. 39.—Haploid chromosome complements selected from representative species of each of the subsections of *Calochortus* and drawn approximately to scale. Numbers indicate the homologies suggested.

result containing 13 instead of the usual 14 chromosomes. From these cells a branch, or branches, might develop and produce flowers containing stamens and pistils. During the prophases of sporogenesis, synapsis would in all probability occur between the translocated chromosome and chromosomes 2 and 6 homologous to it. Two of the four spores resulting from the meiotic divisions would carry the



Behavior in meiosis

FIG. 40.—Diagram illustrating possible occurrence of fusion and translocation between chromosomes 2 and 6 of *C. luteus* to form the translocated chromosome of *C. superbus*, with accompanying reduction in number of chromosomes.

translocated chromosome, with a total of 6, while the other two spores would contain the 7 unchanged chromosomes. Since only the attachment constriction and the small knob have been eliminated in forming the translocated chromosome, it is probable that the spores containing the new type chromosome would be viable. Union between two gametes, each containing the translocated chromosome, would result in the production of a zygote and subsequently a plant homozygous for the translocation, and with a change in chromosome number from the original 14 to 12. The probable occurrence of such

a change is in essential accord with the dislocation hypothesis of NAVASHIN (4). That this process may actually have occurred is supported by a comparison of the haploid complement of *C. luteus* (fig. 39*F*) with that of *C. superbis* (fig. 39*K*).

An increase in the number of chromosomes may also result from the addition of one or more centromeres combined with appropriate "dislocation," as has been postulated also by NAVASHIN. According to this theory, an extra chromosome is introduced by non-division, non-disjunction, or some other aberration in nuclear division, so that the somatic cells of an individual contain three chromosomes of one kind (a trisome). While all the centromeres remain intact, the materials of one member of the trisome are replaced through elimination and translocation by the materials of one (or more than one) non-homologous chromosome, so that the materials of the latter become associated with two centromeres instead of one. Through disjunction at meiosis the normal homologue of the contributing chromosome is segregated out and gametes with the new chromosomal constitution are formed. Union of two such gametes would result in an individual with an extra pair of chromosomes but with the same assemblage of chromosomal materials. The chromosomes would be able to function normally in both mitosis and meiosis. Thus species with higher numbers might arise from one or more containing 14, and from the evolved ones others with still higher numbers might develop.

Evolution of species with numbers greater than 14 may possibly have occurred also through segmentation of chromosomes whose attachment constrictions are in a median or submedian position, provided the break occurs through the centromere so as to provide functional portions of it to both arms of the segmented chromosome. Both arms might then behave normally in mitosis. Again should this process occur during an early stage in embryogeny, a branch, or branches, might develop flowers containing the fragmented chromosome or chromosomes. During sporogenesis the two fragments would likely pair with the respective homologous arms of the non-segmented chromosome, and as a result of meiotic divisions produce two spores containing an "extra" chromosome and two unchanged spores. Union of two gametes containing the extra chromosomes

would result in an offspring containing two more chromosomes than were present in the parents but with unchanged genetic composition. Subsequent mutations might cause sufficient morphological character changes as to constitute species differentiation, and to effectively prevent cross breeding with the original parental species.

Some evidences for the segmentation hypothesis as presented in the preceding paragraph are based upon a comparison of the chromosome complements as shown in figure 39. For example, should chromosome 7 of *C. luteus* (fig. 39 I') break through its centromere, it would result in a chromosome configuration similar to that of *C. nuttallii* (fig. 39 K). Thus a 16 chromosome form might arise from a 14 form. The same type of fragmentation may account also for the higher chromosome numbers, including the 18 and 20 forms. McCLINTOCK (3) has reported one case in *Zea mays* in which the break occurred through the attachment region, so that the two portions of the fragmented chromosome possessed functional parts of it.

Summary

1. The species of *Calochortus* occur naturally only in western North America. A total of twenty-eight species and five varieties representing fourteen species in the *Eucalochortus* section, thirteen species and five varieties in the *Mariposa* section, and one species in the *Cyclobothra* section have been investigated.

2. Somatic numbers of 12, 14, 16, 18, 20, 21, 28, 32, and 40 have been observed, with corresponding gametic numbers of 6, 7, (8), 9, 10, (14), (16), and 20. (Numbers in parentheses have not been observed but are inferred from the somatic numbers.)

3. A rearranged classification, based upon both cytological and morphological criteria, is presented as outlined by MR. MARION OWNBEY. In this classification all species with a basic number of 10 chromosomes and having capsules winged on the angles are placed in the *Eucalochortus* section, while those species with some basic number other than 10 and having capsules not winged are placed in the *Mariposa* section, except *C. plummerae*, with $n = 9$ chromosomes, which for other reasons is placed in the *Cyclobothra* section. The chromosome numbers and their morphology, together with the

morphological characters of the plants, have made necessary this rearrangement.

4. Seven is suggested as the basic number of chromosomes for the *Mariposa* section, and perhaps for the genus also. One species, *C. superbis*, with 12 somatic chromosomes, may have been derived from *C. luteus* through fusion and translocation between two chromosomes of the latter.

5. Evolution of species with somatic numbers greater than 14 may have resulted from the addition of one or more centromeres combined with appropriate "dislocation," as postulated by NAVASHIN.

6. It is also possible that fragmentation of medianly or submedianly constricted chromosomes through the centromere so as to give each of the arms a functional portion of the centromere may have resulted in increasing the chromosome numbers, and in the formation of new species.

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DISTRIBUTION AND HYBRIDIZATION OF VERNONIA IN MISSOURI

CORA SHOOP STEYERMARK

(WITH SEVEN FIGURES)

Introduction

This study has been undertaken with the aim of determining, as far as possible, the species of *Vernonia* in Missouri; the hybridizing possibilities of the species; and the extent to which characters persist if pollination is controlled. Three lines of procedure were followed, extending over a period of six years. (1) Dried specimens were studied; (2) plants were studied in their natural habitats; (3) plants were grown from seeds of assumed species and hybrids of different vernonias.

1. More than 1000 dried specimens from seventy-five counties of Missouri were studied and compared in the process of separating species with their variations from other species. Specimens were found which resembled two species so closely that it was a question in which group to place them; these were classed as hybrids. In case of minor variations from the species type, which left no doubt as to where the plant belonged, the specimen was given the species name. It was found that DANIELS' (3) eight new species were hybrids or variations from the species *V. baldwini*, *V. missurica*, and *V. altissima*.

In the herbarium of the Missouri Botanical Garden several hundred specimens of *Vernonia* from other states in the United States, Mexico, and Central and South America were studied for the purpose of determining the ancestry of our own species; to see what characteristics persisted during migration toward Missouri; and to what extent past structures might have influenced characters of our present species.

2. More than 1000 plants of *Vernonia* were studied in the field in their natural habitat. Special attention was given to altitude, soil moisture, soil type, and sunshine and shaded areas to determine if

possible what bearing these had upon the species, and to what extent they caused variations.

3. Seeds from hybrids, and from plants which because of radically distinct characteristics and wide distribution would seem to be new species, were planted in garden plots and observed for from one to three generations.

From this study it is concluded that, for Missouri, only the five species described in the key are distinct. *V. interior* does not appear to be distinct enough to justify inclusion as a species; it needs more genetical and cytological investigation.

Key to Missouri species of *Vernonia* Schreb.

Heads large, 8–16 mm. high and 10–18 mm. broad; number of achenes 60–115; involucre bracts tipped with long filiform appendages; leaves chiefly glabrate above and below . . . *V. crinita*

Heads small, 4–8 mm. high and 5–9 mm. broad; achenes 14–60.

Leaves glabrate or with pubescence on veins of lower surface.

Involucre bracts appressed, chiefly acute.

Inflorescence a fastigiate cyme; leaves deeply pitted¹ beneath . . . *V. fasciculata*

Inflorescence a panicle; leaves not pitted . . . *V. altissima*

Leaves from pubescent to tomentose on lower surface.

Involucre bracts acute, recurved, resinous on each side of prominent midvein.

Bracts broadly acute or narrowly acuminate, abruptly or only slightly recurved . . . *V. baldwini*

Involucre bracts chiefly obtuse, appressed, midvein not prominent . . . *V. missurica*

Observations

ECOLOGICAL VARIATION AND DISTRIBUTION

According to GLEASON (4) there is no correlation between structure and habitat. Field observations indicate in a general way that the typical xerophytic *Vernonia* grown in the sun has thicker and more tomentose leaves.

¹ Dot-like depressions.

Individual plants under observation for six consecutive years in the garden plot showed from year to year considerable variation in height of plant, length and width of leaves, color of stalk, leaves, and pappus (table 1). These variations were due to the only variant factors—rainfall, number of days of sunshine, and possibly temperature.

YAPP (12) regarded the action of light and water as a factor in determining the structure of “sun” and “shade” leaves. He found

TABLE 1
VARIATION BETWEEN PARENT PLANT, *V. CRINITA*, AND SEEDLING *F₁*
AND VARIATIONS OF *F₁* IN SUCCEEDING YEARS

V. CRINITA	HEIGHT (FEET)	INVOLUCRE		LEAVES		SCALES	CHARACTER OF STEM AND LEAF
		HEIGHT (CM.)	WIDTH (CM.)	WIDTH (CM.)	LENGTH (CM.)		
1884 P ₁ ...	3	0.6	1.4	1-1.4	12-14	Cauline, not exceeding height of involucre	Smooth and purple tinted
1884 <i>F₁</i>							
1935....	5½	1.2	2	2	12-15	Scale append- ages of typical <i>V. crinita</i> long and curl- ing, much exceeding height of involucre	Smooth and purple tinted
1936....	5½	1.1	2	0.5-0.7	10-13		
1937....	6	1.4	2.1	0.8-1.5	11-13		

that the degree of pubescence followed closely the mean curve of evaporation and light intensity in both space and time. MAXIMOV (6) stated that “a decrease of soil moisture induces changes in leaf structure in the direction of increased xeromorphy.” He also found that in plants growing in deep shade the upper and lower leaves show less difference in venation and pubescence than when growing in sunshine. POOL (9) stated that the xerophytic leaf is thick and the stoma-bearing surface hairy.

V. crinita, and *V. crinita* × *V. missurica* of the Schwieder habitat, 8 miles east of Steelville, Missouri, had decided tomentose lower leaf surface, stems, and bracts. These plants, which grew along a spring

branch, were rooted in swamp much of the summer, yet exposed to sunshine all day. Seeds taken and grown in the garden plot produced typical *V. crinita*.

The general order of ecological distribution of the common species found in the Ozarks, beginning with lowlands and ascending in altitude, is: *V. crinita*, *V. altissima*, *V. missurica*, and *V. baldwini*.

V. crinita was collected from forty-four counties south of the Missouri River. It is definitely confined to the lowlands of the Ozarks. PALMER and STEYERMARK (8) found that *V. fasciculata* occurred on prairies, meadows, and alluvial soils along streams of twenty-eight counties, chiefly in northern and central Missouri. *V. altissima* also occurred in lowlands in twenty-eight counties, chiefly in eastern, central, and southern Missouri. *V. missurica* was found distributed generally in forty-four counties, occurring very commonly in the northeastern part of the state. *V. baldwini*, collected in fifty-four counties, apparently has the widest distribution. It is least commonly found in northwestern Missouri. Herbarium specimens labeled *V. interior* were examined from twenty-five counties.

Of interest is the absence of all vernonias above a certain elevation in Dent and Shannon counties along highway 19. The upland soil along most of this highway is Clarksville stony loam, which dries very quickly. From about 5 miles north of Salem to 10 miles south, the upland soil is of Hanceville loam. This soil is loose, thin, and of low fertility. Forests and much vegetation common to lower levels were growing to the summit of the highest points, but *Vernonia* ended at definite levels on either slope of the hills, owing probably to a lack of soil moisture.

The H ion concentration of the soil may have some influence in the distribution of *Vernonia*; however, only one test was made. Several samples of soil were tested from different parts of a 4-acre field 2 miles east of Sigo in Dent County, where *V. crinita* was the only species growing. The test showed no trace of acidity. The reaction ranged from neutral to slightly alkaline. KURZ (5) found a correlation between H ions and the distribution of a number of species, but he examined no *Vernonia*.

One colony of white *V. altissima albiflora* Raf. was found 10 miles west of Steelville near the Meramec River. The colony consisted of

twelve separate clumps, in some places 15 feet or more apart. The flowers are pure white, pappus tan or golden, stems, leaves, and bracts pale green and consistently smooth. Also one white-flowered plant of the same species was found 2 miles east of Columbia, Missouri, in a highland forest, in August, 1932. It had the characteristic smoothness, while all other plants of *Vernonia* growing near were very pubescent forms of *V. altissima*.

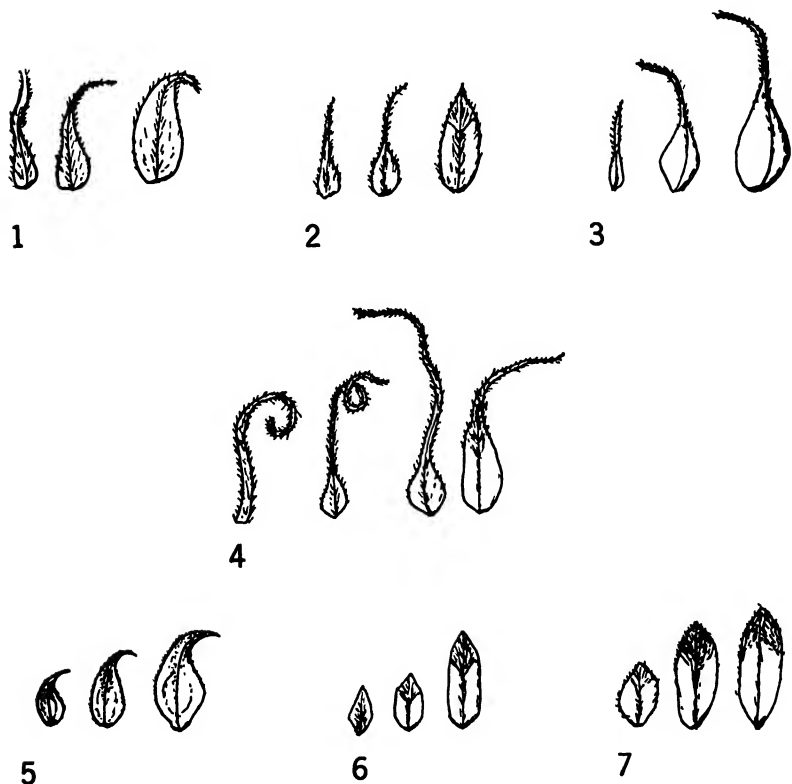
BUSH (2) made a study of the mound flora of Atchison County during August, 1895. He reported seventy-nine species, but did not mention *Vernonia* for that county.

EFFECTS OF NATURAL GROWING

Uniformity of habitat, including climatic, edaphic, and biotic factors, tends to produce plant communities, according to NICHOLS (7), in which species within a certain genus take on similar characteristics. TURESSON (11) stated, "Similarity in growth form shown by different species when inhabiting similar habitats is merely a modification induced by similar habitat factors, while the similarity in growth form of a plant inhabiting very extreme habitats is held to be of hereditary nature." The similarity of different species of *Vernonia* in each of the habitats mentioned for Missouri is to a greater extent due to crossing than to edaphic factors. Some examples illustrate this position. A colony of *Vernonia* in southern Howell County, considered a hybrid between *V. baldwini* and *V. crinita* (fig. 1), had most of the characteristics of typical *V. baldwini* (fig. 5), but possessed the long, curling, filiform scale appendages of typical *V. crinita* (fig. 4). These plants were growing on a high prairie, a typical *V. baldwini* habitat. *V. crinita* was growing several miles away along creeks, and *V. baldwini* was found among the assumed hybrids. *V. baldwini* × *crinita* of figure 1 had the general appearance of *V. baldwini* at a distance, with an involucre height of 5 mm. and a width of 8 mm. The leaves were 3 cm. wide by 10 cm. long, obtuse at the base, with lower leaf surface very pubescent. Other similar hybrids of *V. baldwini* and *V. crinita* were found 8 miles east of Steelville, 5 miles west of Steelville, at Steelville, and in Texas County. In no place north of the *V. crinita* region was any plant found

bearing *V. crinita* characteristics. Figure 2 shows a cross of *V. crinita* with *V. altissima*. Figure 6 shows bracts of typical *V. altissima*.

V. baldwini and *V. altissima*, with fastigiate cymes and leaves and bracts closely resembling *V. fasciculata*, were found frequently in



FIGS. 1-7.—Involucral scales, from smaller to larger: Fig. 1, *V. baldwini* × *V. crinita* from Howell County; fig. 2, *V. altissima* × *V. crinita* from 2 miles east of Sligo; fig. 3, *V. crinita* × *V. missurica* from 8 miles east of Steelville; fig. 4, *V. crinita* herbarium specimen no. 1056; fig. 5, *V. baldwini* no. 1120; fig. 6, *V. altissima* no. 1004; fig. 7, *V. missurica* no. 1138.

the northern part of the state. Twenty-five specimens examined from Buchanan and Andrew counties showed hybridization between *V. fasciculata* and *V. baldwini*.

Where *V. missurica* is the dominant species, there is a tendency for the other species of *Vernonia* to hybridize with it.

In the Ozarks, plants of every other species of *Vernonia* common to the state, except *V. fasciculata* which does not occur in that region, were found with resemblance to *V. crinita* in either or both of the following: a marked increase in length of scale appendages and increased size of head; or in type of leaf structure. This gives evidence that *V. crinita* has crossed with the other species, producing wide variations. The typical *V. crinita* follows the alluvial and gravel soils of river and creek bottoms at an altitude between 500 and 750 feet. Evident crosses of *V. crinita* with *V. missurica* are shown by bract variations (fig. 3). Both involucre and leaves tend toward a median between the species crossed. Figure 7 shows bracts of typical *V. missurica*.

TABLE 2

AVERAGE NUMBER OF ACHENES PER HEAD IN TYPES AND HYBRIDS OF VERNONIA

VERNONIA	V. FAS- CICULATA	V. ALTIS- SIMA	V. IN- TERIOR	V. BALD- WINI	V. MIS- SURICA	V. CRI- NITA
fasciculata.....	(20)*
altissima.....	(22)	52
interior.....	28	(28 8)	29.5
baldwini.....	(29)	54
missurica.....	36	(41)	40
crinita.....	48	52	(85)

* Types given in brackets.

Many species and hybrids were examined to obtain the average number of achenes per head in an attempt to determine what relationship existed between the two supposed parents and the hybrid. Table 2 shows the results obtained.

GROWTH FROM SEEDS

On October 3, 1931, twenty-five plots in the garden were planted with seeds from twenty-five specimens of *Vernonia*, including types and variations of *V. crinita*, *V. baldwini*, *V. interior*, *V. altissima*, and *V. missurica*. Sixteen pots were planted in the house November 1, 1931. Of these sixteen, half the seeds were treated for 2 hours with concentrated sulphuric acid and half were left untreated. On November 28 of the same year, twelve plants appeared in treated pot no. 1026, *V. crinita*. About the same number of plants appeared

in untreated pot no. 1027, *V. crinita*. March 17, 1932, five seeds germinated in treated pot no. 1035, *V. altissima*. April 10, one seed germinated in treated pot no. 1037, *V. baldwini* (DANIELS' new species, *V. flavipapposa*, with the golden pappus). May 2, one plant appeared in outdoor plot no. 1007, *V. crinita*, and also five seeds germinated in outdoor plot no. 1012, *V. baldwini* (*V. flavipapposa* Daniels). June 1, one plant appeared in treated pot no. 1029, *V. baldwini*, oval-leaved form.

Seeds were used from plants collected in the field without any attempt to prevent crossing. Most of the fruits which failed to germinate were collected in August and probably were not mature. May 4, 1932, the potted plants were transplanted to the garden.

Of the seven plants raised from the one head of no. 1026, *V. crinita*, six seedlings closely resembled the parent plant. Individual no. 5 F₁ of no. 1026 P₁ showed an evident cross with *V. altissima*. The involucre of the parent *V. crinita* measured 8 mm. high and 14 mm. wide with filiform appendages more than equaling the height, while the involucre of no. 5 F₁ measured 6 mm. high and 7 mm. wide, with slender, straight, erect appendages barely reaching the rim of the involucre. The leaves of the seedling exceeded the parent in both length and width (table 3); otherwise there was close resemblance. *V. altissima* was growing on the plot from which no. 1026 P₁ was taken.

The scale appendages of individual no. 7 F₁ of no. 1026 P₁ were longer and more curled than those of the parent, completely enveloping the head until in full blossom.

V. baldwini, no. 1037 P₁, with golden colored pappus and lanceolate, tapering leaves, produced an F₁ with tawny pappus, showing decided purple in parts not exposed and blunt ovate leaves 4.5×9.5 cm., illustrating another cross in the field. An oval-leaved *V. baldwini* was growing in the plot where no. 1037 P₁ was collected.

The one plant from seeds of *V. baldwini*, no. 1012 P₁, described by DANIELS as *V. flavipapposa*, resembled the parent in every respect the first year; however, the pappus color varied from year to year. In 1933 the color was a dull purple near the base, becoming tawny with age. In 1936 the color took on the glossy golden shade of the parent. In 1937 the pappus on each of the forty-seven stalks from

TABLE 3

VARIATION BETWEEN PARENT PLANT AND SEEDLING FLOWER
IN EACH SPECIES, GROWN SEPTEMBER 10, 1932

VERNONIA		A-CHENE	PAPPUS	HEADS	INVOLUCRE (MM.)	SCALES	STEM	LEAVES	
baldwini 1037	{	P _i	35	Golden	130	height 5 width 6	Typical	Green	Lanceolate 2.5×9.5 cm.
		F _i	30	Tawny	90	height 5 width 6	Typical	Green	Ovate, blunt 4.5×9.5 cm.
baldwini 1012	{	P _i	25	Golden	125	height 5 width 6	Typical	Green	Lanceolate 1.7-2×4-10 cm.
		F _i	25	Golden	72	height 5 width 6	Typical	Green	Lanceolate 2-3.5×3-10 cm.
crinita 1007	{	P _i	85	Purple	29	height 9 width 16	Filiform	Green	Finely serrated 1×10 cm.
		F _i	84	Purple	63	height 9 width 16	Filiform	Green	Coarsely serrated 2.5×14 cm.
crinita 1026	{	P _i	105	Tawny	50	height 8 width 14	Filiform	Red	1.3×14 cm.
		(1) F _i	80	Tawny	21	height 7 width 14	Filiform	Green	1.7×10 cm.
		(2) F _i	?	Tawny	25	height 7 width 14	Filiform	Green	2-3×16 cm.
		(3) F _i	?	Tawny	15	height 7 width 14	Filiform	Green	2-3×16 cm.
		(4) F _i	?	Tawny	13	height 7 width 14	Filiform	Green	2-3×16 cm.
		(5) F _i	?	Tawny	26	height 6 width 7	Erect Exceeding the head	Red	2.5×16 cm.
		(6) F _i	?	Tawny	16	height 8 width 14	Longer, more prominently curled than P _i	Green	1.7×14 cm.
		(7) F _i	?	Tawny	38	height 8 width 14	Filiform	Green	1.5×15 cm.
altis- sima 1031	{	P _i	26	Tawny	500	height 5 width 6	Typical	Red	Smooth 2-3×15 cm.
		(1) F _i	?	Tawny	85	height 5 width 6	Typical	Red	Puberulent
		(2) F _i	?	Tawny	92	height 5 width 6	Typical	Red	Smooth 2×12 cm.
		(3) F _i	?	Tawny	62	height 5 width 6	Typical	Red	2×13 cm.

the original rhizome was a dull straw color. As seen through the low power of a compound microscope, the bristles appear barbed, with varying numbers of erect, more or less appressed barbs. The purple pappus is more densely barbed than the golden. The pappus of this parent plant is the glossy golden color which caused DANIELS to separate it as a distinct species. No. 1012 P₁ was taken from 2 miles east of Sligo, the only individual with this type of pappus within a quarter of a mile of the plot. *V. baldwini*, with this conspicuous and attractive golden pappus when dry, is found in the approximate ratio of 1 plant to 500 of the typical *V. baldwini*. Of the specimens observed in the herbarium of the Missouri Botanical Garden, the same type of pappus was found in one specimen of *V. altissima*, one of *V. noveboracensis*, and one of *V. glauca* from other states.

Herbarium specimens of the ancestors of our species from South America, Central America, Mexico, and the West Indies which were examined in the Missouri Botanical Garden showed very little variation in pappus color, ranging from pale cream to golden. Only two exceptions were noted: one specimen from Brazil had a nearly black pappus and another from the same country had a tawny pappus. It seems that the purple pappus is characteristic only of species in the United States. The occasional occurrence of the golden pappus here might indicate that it is a reversion to the color of the ancestral species of South America, pollen from one plant heterozygous for that color having fertilized the megagamete of another plant also heterozygous for the same ancestral color of pappus. Another possibility exists, that of mutation. In either case DANIELS' *V. flavipapposa* certainly crosses with such ease that it does not perpetuate itself as a species.

V. baldwini, no. 1012 F₁, showed also some leaf variation in size in succeeding years. On the parent plant, no. 1012 P₁, leaf measurements halfway down the stem were 1.7-2×4-10 cm. On F₁ they were: in 1932, 2-3.5×3-10 cm.; 1933, 4-5×12 cm.; 1936, 4-5×9 cm.; 1937, 5-5.5×14-15 cm.

Individual F₁ of parent 1029, *V. baldwini*, with oval leaves, resembled the parent closely in leaf form. However, it failed to live to maturity.

Since the seeds of no. 1026 P₁, *V. crinita* (table 3), all came from

the same head, and since the seven seedlings of this parent had practically the same environment, the resulting variations must be genetic.

In September, 1934, seeds were collected from specimen *V. crinita*, no. 1884, with short cauline appendages and narrow leaves. These were planted in November of the same year in the garden plot. The parent plant, growing at the base of a west-facing, rocky hill, was only 3 feet high. Other typical *V. crinita* were growing along the creek bed 10 feet away. All fourteen plants of the F_1 generation, with their large heads and long curling scale appendages, showed very little similarity to the small head and short acute appendages of the parent plant. It will be noted in table 1 that while the height of F_1 and the size of its involucre nearly doubled that of P_1 , the leaves of F_1 were narrower. Hence in every respect the F_1 tended to approach the typical *V. crinita*.

May 3, 1934, sixty-five plants appeared from seeds gathered October 19, 1933, from a colony of *V. altissima albiglora*, no. 1883, and were planted in the garden November 1, 1933. Detailed records were kept for sixteen of these progeny. Out of the sixty-five seedlings, fourteen resembled the parent in every respect, with pure white flowers, smooth yellow-green leaves, stems, and heads. These plants were also very consistent in leaf size, shape, and serrations. The shape of the head varies somewhat from *V. altissima* in being slightly more elongated and more pointed. *V. altissima albiglora* can readily be distinguished from other *V. altissima* in the garden plot before bud color begins to show. Twenty-six other individuals of the F_1 had green heads, leaves, and stems, with purple showing only in the notches of the leaves. The remaining twenty-five were definitely purple tinted in all parts (table 4). In the purple forms there was wide variation in pubescence from smooth to heavily pubescent. Leaves varied from 10 to 25 cm. in length and from 2 to 5 cm. in width. Pappus color varied from tan to purple.

Heads of white-flowered individual no. 4 F_1 of no. 1883 P_1 were covered with waxed bags during flowering to prevent cross pollination. Seeds from these heads were planted November 20, 1934, in the garden plot. Of the four seeds which germinated, two of the F_2 were typical of the white-flowered parent and two were light green

TABLE 4

CHARACTERISTICS OF F₁ AND F₂ OF V. ALTISSIMA ALBIFLORA 1883 P₁

V. ALTISSIMA ALBIFLORA	COLOR OF FLOWER	COLOR OF LEAF, STEM, AND BRACTS	LEAVES		CHARACTER OF LEAVES, STEM
			WIDTH (CM.)	LENGTH (CM.)	
1883 P ₁	White	Light green	1.5-2	10-14	Smooth throughout
1883 F ₁ No. 4	White	Light green	2.5	10-12	Smooth throughout
1883 F ₂ No. 17	White	Light green	2-2.1	10-13	Smooth throughout
1883 P ₁	White	Light green	1.5-2	10-14	Smooth throughout
1883 F ₁ No. 10					
1935	Purple	Dark green with much purple	3-4	17-20	Very pubescent
1937	Purple	Dark green with much purple	3-5.5	18-25	Very pubescent
1883 F ₂ No. 20	White	Yellowish green	2-3	12-15	Smooth throughout
No. 21	Purple	Dark green with much purple	2.5-3.5	16-18	Pubescent
1883 P ₁	White	Light green	1.5-2	10-14	Smooth throughout
1883 F ₁ No. 11	Purple	Light green with purple only in leaf notches	3-3.5	18-20	Smooth leaves
1883 F ₂ No. 19	Purple	Light green with purple only in leaf notches	4.5-6	18-24	Pubescent
1883 F ₁ No. 1	White	Light green	2.5-4	12-15	Smooth throughout
1883 F ₁ No. 2	Purple	Green with much purple	2.3-3	15-18	Smooth
1883 F ₁ No. 3	Purple	Dark green with much purple	3-5	20-25	Very pubescent
1883 F ₁ No. 6	Purple	Purple in notches of leaves	2.5-3	10	Pubescent
1883 F ₁ No. 7	Purple	Light green	2.5-4	15-19	Very pubescent
1883 F ₁ No. 12	Purple	Dark purple	1.5-2	12-14	Slightly pubescent
1883 F ₁ No. 13	Purple	Dark purple	2-3	12-15	Smooth
1883 F ₁ No. 15	White	Light green	2	12-15	Smooth
1883 F ₁ No. 16	Purple	Dark purple	2-4	17-22

in coloring throughout, with purple in leaf notches and purple flowers. Likewise the heads of purple-flowered individual no. 10 F_1 of no. 1883 P_1 were covered. Seeds from these produced an F_2 generation of two typical white and fourteen dark purple individuals.

Seeds from covered individual no. 11 F_1 , with light coloring, produced eight F_2 individuals very similar to the parent.

The common occurrence of *V. crinita* × *V. missurica* (fig. 3) with rather constant characteristics, found over widespread areas where *V. crinita* and *V. missurica* were present, would seem to warrant its description as a species. Experiments in growing succeeding generations from this hybrid, however, prove that the characters which set it aside as new are not transmissible.

While species grown in pots by BOHN (1) failed to flower the same year the seeds germinated, the seedlings listed in this report flowered from four to six months after germination.

Summary and conclusions

1. More than 1000 dried specimens of species of *Vernonia* from seventy-five counties of the state of Missouri were studied and compared in the process of separating species with their variations from other species and in disposing of hybrids. It was found that DANIELS' (3) eight new species were hybrids or variations from the species *V. baldwini*, *V. missurica*, and *V. altissima*.

2. In the herbarium of the Missouri Botanical Garden several hundred specimens of *Vernonia* from other states in the United States, Mexico, and Central and South America were studied for the purpose of determining to what extent past structures might have influenced characters of our present species.

3. On the basis of field characteristics, *V. interior* did not appear separable from *V. baldwini*. Recent investigations have led to its classification as *V. baldwini* var. *interior* Schubert (10).

4. More than 1000 plants of *Vernonia* were studied in the field in their natural habitats. Special attention was given to altitude, soil moisture, soil type, and sunshine and shaded areas, to determine if possible what bearing these had upon the species, and to what extent they caused variations. It was found that minor characteristics such as size, texture, and pubescence of leaf, pubescence of stem,

number of heads, or pubescence of bracts, are influenced to some extent by ecological conditions; but these characteristics are not transmissible.

5. Some species of *Vernonia* are confined to specific regions in the state.

6. The experiments in growing the species led to four conclusions:

(a) Species bloomed the same year they germinated in outdoor plots.

(b) Some plants with wide distribution which looked like new species in the F₁ generation appeared as the typical species which the parent most closely resembled, indicating that the variation was brought about in the first place (1) by environment after the seed had germinated; (2) by somatic modifications brought about by the environment; (3) by a chromosomal variation caused by environment; or (4) by crossing in the field, but not transmissible. Such experiments show that field characteristics alone are not always a safe guide in naming a new species.

(c) Seeds from parents true to type produced some individuals true to type and some plants that indicated crossing in the field.

(d) *V. altissima albiglora*, if field crossing with *V. altissima* is controlled, will produce the usual genetic ratio of pure *V. altissima albiglora*.

7. The results of the three lines of investigation have led to the conclusion that five distinct species of *Vernonia* are rather constant for the state: *V. crinita*, *V. baldwini*, *V. altissima*, *V. missurica*, and *V. fasciculata*, which vary widely. Any one of these five species apparently may cross with each of the other four, producing a wide variety of hybrids.

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ATAVISTIC LEAF FORMS OF VARIOUS SPECIES OF TREES

ELIZABETH S. OLIVER

(WITH TWENTY-THREE FIGURES)

Introduction

Studies of atavistic leaves have been made by VON ETTINGSHAUSEN and KRAŠAN (2) and by NOÉ (19). The writer plans to carry the work further, making a study of various additional genera.

It is generally known that if the leaves of trees or shrubs are destroyed, after a time new leaves appear which do not exhibit the characteristics of the normal leaf. In like manner leaves of adventitious shoots and very young sprouts exhibit atypical leaves. It is believed that such leaves represent a reversion to an ancestral type. With this in mind, leaves of *Populus tremuloides* Michx., *Corylus americana* Walt., *Tilia americana* Linn., *Ulmus americana* Linn., and *Platanus occidentalis* Linn., were collected and studied. In each case the normal leaf form was determined and the amount of variation typical to the normal leaf noted. Then atypical leaves were studied and compared with fossil species, Cretaceous through Tertiary in age. Pleistocene species were felt to be too recent for such comparative use. .

Observations

POPULUS TREMULOIDES MICHX.

Considerable variation in leaf form was found among the normal leaves of this species. After a survey of such variation, leaves believed to be atavistic were studied. These were collected from three sources: (1) shoots which had sprung up after the tree had been cut down; (2) young sprouts; and (3) those resulting from artificial conditions; that is, the first, normal leaves were destroyed the latter part of May and the new leaves which resulted in July were collected for study. All these leaves were found to be atypical and therefore are believed to be atavistic. Comparison was then made with fossil species of *Populus* from the Tertiary and Cretaceous of North

America. Many fossil species were too fragmentary to attempt comparison but showed great similarity to the living atypical leaves. Only fossil leaves which are believed to be duplicated by living ones are included in the following list. Seven fossil species, all Tertiary in age, were found represented in the atypical leaves of the one living species, *P. tremuloides*.

Populus arctica Heer (10)

KNOWLTON (3) states that the species is greatly in need of critical revision. The species was studied as described in the literature, with no attempt at revision. It has a great variety of forms, having margins undulate to dentate to entire. No leaves of *Populus tremuloides* were found having entire margins, but some very nearly approximated those fossils figured with dentate margins. No living specimen has so cuneate a base as the fossil species, but aside from this point some living atavistic leaves resemble the fossil in every detail.

Populus amblyrhyncha Ward (21)

The distinctive character of the leaf of this species is its long blunt apical lobe and the fact that the lateral nerves curve and unite with the midrib. These characters are represented in several leaves of *Populus tremuloides* collected from adventitious shoots formed when a small tree was cut down. The living species differ in one feature: the innermost laterals do not unite with the midrib above the base of the leaf. It is believed that the leaf figured by WARD (24) might show the character of the living specimen referred to if the base of the specimen were complete.

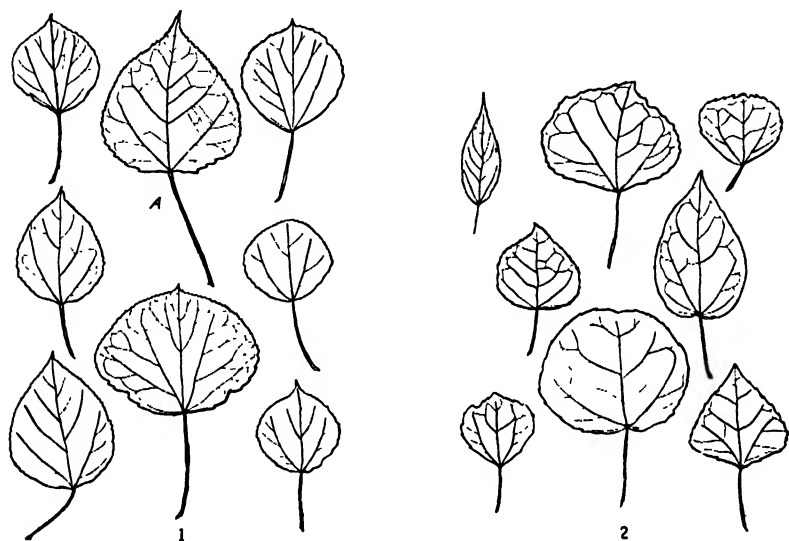
Populus cuneata Newb. (16, 18, 23)

The leaf of *Populus cuneata* is wedge-shaped, obovate, has an obtusely pointed apex, is irregularly dentate, and has three main veins which terminate in the apical portion. The living leaf referred to this species is one which grew on the tip of a branch after the first leaves had been destroyed. On the living leaf the two main secondaries originate above the base, differing at this point from the representative *P. cuneata* with which it is compared. However, other representative leaves of *P. cuneata* have the character represented in the *P. tremuloides* leaf. Also the apex of the living leaf is eaten off,

but the apexes of other leaves on the tip of the same shoot are blunt, greatly resembling the characteristic *P. cuneata* apex.

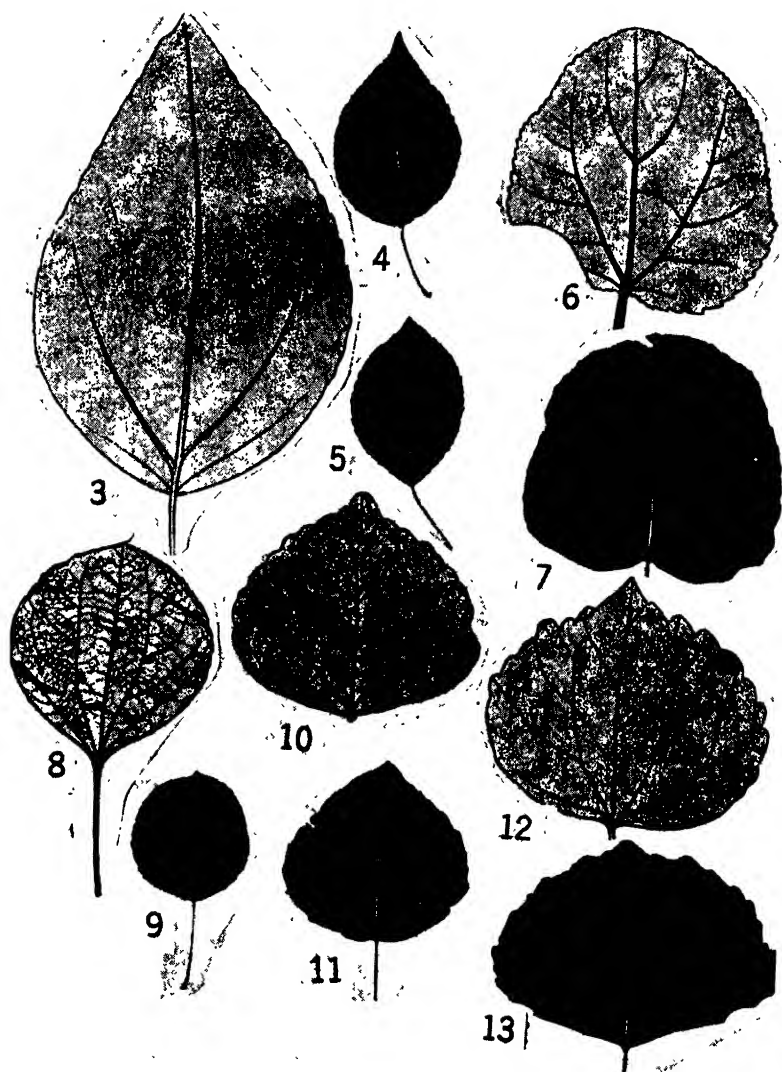
Populus eotremuloides Kn. (4, 6)

KNOWLTON has already referred leaves of this fossil species to those of the living *Populus tremuloides*, finding their equivalent in leaves of a young seedling collected in Utah. One small difference



FIGS. 1, 2.—Fig. 1, typical leaf (A) of *Populus tremuloides* and normal variations. Fig. 2, atavistic leaf forms of same species.

was noted, namely, that the leaf margin of the living species is toothed from the apex to the base while that of the fossil species is entire at the base. KNOWLTON states, "The leaves of this seedling differ somewhat from the normal mature leaves of the species, but it would seem, in view of their evident relationship to the fossil leaves, that they represent a reversion to an ancestral type, or perhaps rather a survival of this type, which disappears in the mature plant" (4). It is believed, therefore, that these seedling leaves represent atavism. In like manner the fossil leaves greatly resemble the majority of second leaves formed on mature trees of *P. tremuloides*. These leaves are also believed to be atavistic. The first leaves were



FIGS. 3-13.—Fig. 3, *Populus eotremuloides*; Figs. 4, 5, *P. tremuloides*, second leaves; Frankfort, Mich. (cf. *P. oetremuloides*). Fig. 6, *P. lindgreni*. Fig. 7, *P. tremuloides*; leaves from adventitious shoots; Lake Ann, Mich. (cf. *P. lindgreni*). Fig. 8, *P. arctica*. Fig. 9, second leaf of *P. tremuloides*; Frankfort, Mich. (cf. *P. arctica*). Fig. 10, *P. amblyrhyncha*. Fig. 11, *P. tremuloides* from adventitious shoot; original tree cut down; Lake Ann, Mich. (cf. *P. amblyrhyncha*). Fig. 12, *P. oxyrhyncha*. Fig. 13, *P. tremuloides*, collected from young shoot; Frankfort, Mich. (cf. *P. oxyrhyncha*).

severed from the trees about May 28 and the new leaves which appeared have the characters of *P. eotremuloides*. They are very much smaller than those of the fossil species, owing to drought, but the main characteristics of the fossil species are well represented.

Populus lindgreni Kn. (5, 7)

A great number of leaves approximate this species, but no one of them has all of the characters. Several leaves, however, all from adventitious shoots formed as a result of cutting of the main tree, show all the major characters. One leaf exhibits the circular shape of *Populus lindgreni* and the correct veining and margin, except that the angle between the main secondaries and the midrib is too great. Another exhibits the correct angle between the main secondary but not the correct shape. The margin of the fossil species is toothed, while on all individuals of the living species referred to *P. lindgreni* the base of the leaf is entire. This is the only constant difference between the fossil species and those living leaves referable to it.

Populus oxyrhyncha Ward (22, 26)

One leaf taken from a young shoot was found to have most of the characters of *Populus oxyrhyncha*. The apex is not so acute as that in the figured specimens, and the inner lateral primaries do not curve inward toward the apex as much as do those in the fossil species. Aside from these two points the living leaf fits the description of *P. oxyrhyncha*.

Populus neotremuloides Kn. (9)

The one figured specimen of *Populus neotremuloides* is very fragmentary. KNOWLTON referred it to the young vigorous sprouts of *P. tremuloides*, finding the characters of the fossil well represented in this living species. Here the fossil is also compared with a large leaf from a young sprout. It does vary from the fossil description, which says the margin is entire at the base and slightly toothed to undulate on the upper part of the leaf. The margin of the living specimen is very coarsely and irregularly toothed throughout. Also the second pair of main secondaries curves downward to merge with the midrib, and the general venation is not so coarse.

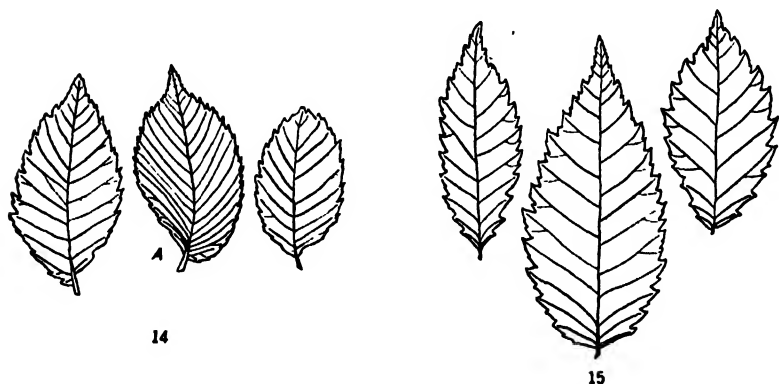
The fragmentary nature of the fossil leaves some doubt as to its

shape and the true nature of the margin. The venation of the fossil and the living leaves is very similar, KNOWLTON having already noted this similarity, referring his species to a leaf of this living species also from a young sprout.

Age of fossil species represented by atavistic forms of the living *Populus tremuloides*:

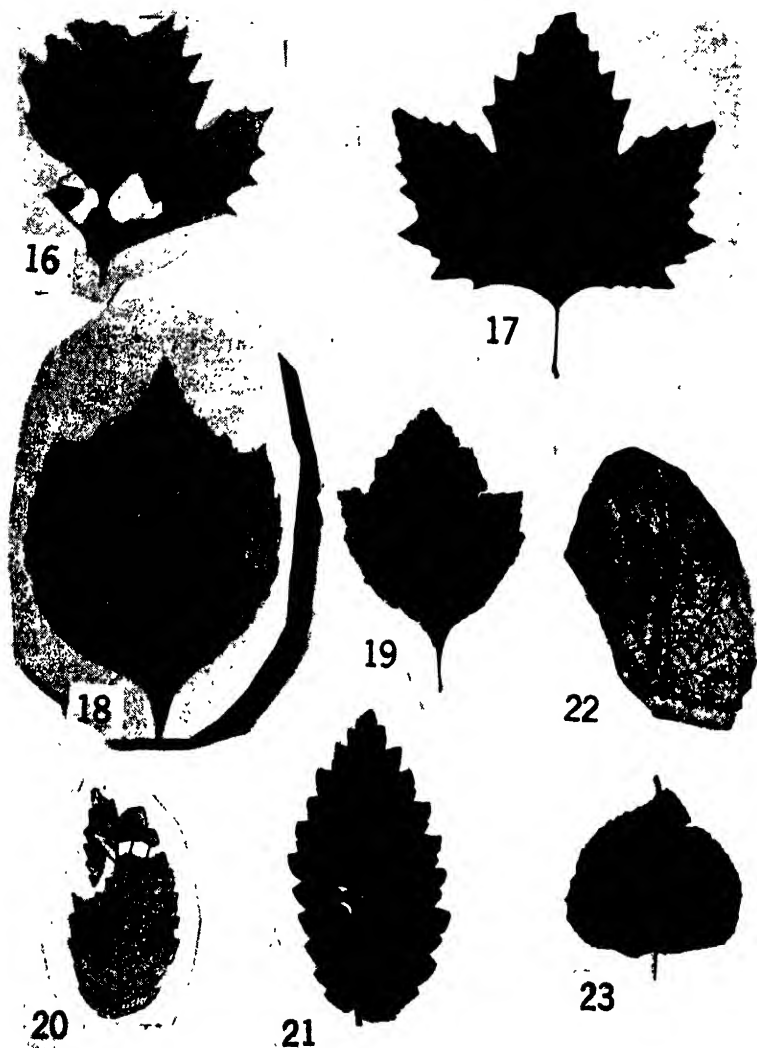
<i>P. arctica</i>	Eocene, Oligocene
<i>P. amblyrhyncha</i>	Eocene
<i>P. cuneata</i>	Eocene, Oligocene, Miocene (?)
<i>P. cotremuloides</i>	Eocene
<i>P. lindgreni</i>	Eocene, Miocene
<i>P. neotremuloides</i>	Eocene
<i>P. oxyrhyncha</i>	Eocene

It may be noted that the preponderance of fossil species to which the atavistic forms of *Populus tremuloides* may be referred occurs in the Eocene. Since there is such a wide variation in the normal leaf



FIGS. 14, 15.—Fig. 14, typical leaf (A) of *Ulmus americana*, and leaf variation on normal shoot. Fig. 15, atavistic leaves of same species.

form of the present *P. tremuloides*, there might be a possibility that the present species is somewhat unstable as to leaf form and thus the atavistic forms revert back farther, representing fossils found in the earliest Tertiary.



FIGS. 16-23.—Fig. 16, *Platanus latior grandidentata*. Fig. 17, *P. occidentalis*; Carleton College nursery, Northfield, Minn. (cf. *P. latior grandidentata*). Fig. 18, *P. raynoldsii*. Fig. 19, *P. occidentalis*; Carleton College Nursery (cf. *P. raynoldsii*). Fig. 20, *Ulmus wardii*. Fig. 21, *U. americana*; leaf from adventitious shoot from base of stump after tree had been cut down; Nerstrand, Minn. (cf. *U. wardii*). Fig. 22, *P. neotremuloides*. Fig. 23, *P. tremuloides*; leaf from young sprout; Lake Ann, Mich. (cf. *P. neotremuloides*).

CORYLUS AMERICANA WALT.

Great variation in leaf form is exhibited in the living species. Leaves which have been damaged by grazing are coarse and very pubescent. Those unhurt seem to be much thinner, less pubescent, and less finely serrate. The veins of the normal leaves are primarily alternate. As the leaves show atavism there is a tendency for veins to become opposite.

Corylus americana, the fossil species, seems to be made up of a great variety of leaf form and venation. Some representatives (27) correspond to the typical leaf of the modern species, while other representatives of the same species, all from the Fort Union flora, are represented in the atavistic types. The normal leaves of the living species characteristically have alternate primary venation, while the atavistic leaves have a tendency toward the primary veins becoming opposite. The primary veins of the fossil species are generally alternate.

All of the variation of leaf characteristics in the fossil leaf species is represented in leaves of the living species.

TILIA AMERICANA LINN.

There is much variation in the form of the normal leaves in the living species. They are five, six, or seven nerved; the base is asymmetrical, deeply cordate to flat, and the apex is acute to acuminate.

Leaves developing as the result of defoliation or from adventitious shoots after the main tree has been cut down are generally larger in size, but there is so much variation among the normal leaves that the increase in size is their major distinction. Generally speaking the smaller leaves are deeply cordate, and with increase in size there is a tendency toward a flat oblique base.

One fossil species, *Tilia populifolia* Lesq., represented in the Fort Union and Florissant floras, was found to have a counterpart in several leaves from adventitious shoots of *T. americana*. The tip of the figured fossil specimen is broken off, but from its description and the figures one is able to compare the fossil and living species.

ULMUS AMERICANA LINN.

Leaves of *Ulmus americana* which were abnormal were collected from shoots which had sprung up around old stumps after the trees had been cut down. The leaves varied in shape and size, but were similar in several major respects. They seem to have lost the characteristic obliqueness of *U. americana*, are coarsely dentate, and are of a thin texture.

Very few of the abnormal leaf types of *U. americana* were found to be represented in the various reported species of fossil *Ulmus*. The reason for this is believed to be that since a major characteristic of the normal leaves of this species is their obliqueness, some fossil species were not recognized as such because of lack of this character.

It is the general contention that plants whose leaves are injured in some way will put out new ones which are usually unlike the normal leaf and which represent a reversion to the ancestral type. If this is true in the case of the *Ulmus* leaves under discussion, there are probably many leaves of the genus present in the fossil record which have never been recognized as such because they do not exhibit the characteristics so well shown in the normal leaves.

The abnormal leaves show a marked similarity to those of *Zelkova ulmoides* Schneider of southwestern Asia and *Zelkova oregoniana* (Kn.) Brown, a Mascall formation, Miocene species. This may mean that *Zelkova* and *Ulmus* had a common ancestor.

PLATANUS OCCIDENTALIS LINN.

Several trees of *Platanus occidentalis* have been planted in the nursery at Carleton College in Northfield, Minnesota. Owing to the rigorous climate, these trees die to the ground each winter and send up new shoots each spring. The leaves vary greatly in size and shape. The large trilobate leaves are found near the tips of the shoots, while the small leaves which have a tendency away from the lobing, are found near the base of the shoots. The leaves from these shoots were collected and compared with the various fossil species of *Platanus*. Five fossil species, which ranged in age from the Cretaceous through the Miocene, were found to be referable to living species.

Geologic ages of fossil species referred to *P. occidentalis*:

<i>P. dissecta</i>	Miocene
<i>P. guillelmae</i>	Eocene
<i>P. haydenii</i>	Eocene
<i>P. latior grandidentata</i>	Cretaceous
<i>P. raynoldsii</i>	Eocene

Platanus dissecta Lesq. (11)

Platanus dissecta exhibits the same variation shown in the modern leaves. According to BROWN's figures (1) the species includes leaves which vary in form from trilobate to unlobed, as do the living leaves. The leaves of the living species agree with *P. dissecta* in all details except that the angle of the secondaries is much greater in the living species. However, there is so much variation in this point, the smaller unlobed modern leaves exhibiting the angle characteristic of the fossil species, that it is believed the living leaves are referable to this fossil species. The living leaves have a cuneate base with the first primaries arising well above it. This characteristic is present in the fossil species but is not constant.

Platanus haydenii Newb. (15)

The atypical leaves of *Platanus occidentalis* under consideration vary in form from deeply trilobate to an almost complete absence of lobing. Those which are lobed generally have a dentate margin, and the primary and secondary veins arise at the midrib, forming a wide angle. The leaves which are not trilobate have a tendency toward a doubly dentate margin and the veins form a much more acute angle with the midrib. The fossil species represents a composite of the two types. While no one leaf can be referred to the fossil leaves figured, the group as a whole has the characteristics of the fossil species—the acute angle of the primary and secondary veins and the simple dentation. NEWBERRY (14) has pointed out that this fossil species might be one of the ancestors of *P. occidentalis*. "In general aspect the species now before us is more like the eastern than the western of our American sycamores, to the former of which it has considerable likeness and may very well have been its progenitor."

Platanus latior (Lesq.) Kn. (12)

The nervation and shape of this fossil species resemble closely some of the atypical leaves of *Platanus occidentalis*. There is one point at which there is a lack of similarity, the margin. None of the atypical leaves exhibited such blunt, simple, widely spaced teeth as characterize the fossil species. LESQUEREUX referred to the great similarity of the fossil species to *P. occidentalis*, and for that reason it is included in this report. "Indeed, comparing the leaves of *P. primaeva* [*P. primaeva* Lesq. = *P. latior* (Lesq.) Knowlton] of the Dakota group with those of *P. haydenii* of the Laramie, those of *P. guillelmae* of the old Pliocene of California, and those of the living *P. occidentalis*, one sees the original type so clearly and distinctly preserved that, overlooking the great difference in age, it would be difficult to separate these into different species" (13). The atypical leaves of *P. occidentalis* show marked similarity to *P. latior grandidentata* (Lesq.) Knowlton, and are probably referable to that species.

Platanus guillelmae Göppert (8, 20)

As already stated, LESQUEREUX (13) remarked on the similarity between the fossil species and *P. occidentalis*. The leaf specimens here under consideration may be referred to some figured leaves representing this fossil species (8), but they are not similar to the one fossil figured as representing the same species which WARD (25) states most nearly resembles the type fossil of the species figured by GÖPPERT.

Platanus raynoldsii Newb. (17)

NEWBERRY describes the general character of the nervation and leaf as a whole as similar to that of *Platanus occidentalis*. The great difference is in the margin, that of the fossil species being characterized by being doubly dentate. Some of the atypical leaves of *P. occidentalis* exhibit the same characteristic, so it is here compared with this fossil species.

Summary

If the normal leaves of trees and shrubs are destroyed, the leaves which subsequently develop are usually atypical. When these

atypical leaves are compared with fossil species belonging to the same genus, a great similarity is noted. The characteristics of the atypical leaves of the living species are found represented in the fossil species, and for that reason the atypical leaves are believed to be atavistic; that is, to represent a reversion to an ancient type present during the growth and development of that species in the past.

This study was made under the direction of Dr. A. C. NOÉ of the University of Chicago, to whom the writer is greatly indebted.

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MAJOR CHANGES IN GRASSLAND AS A RESULT OF CONTINUED DROUGHT¹

J. E. WEAVER AND F. W. ALBERTSON

(WITH NINE FIGURES)

Introduction

A comprehensive research on 135 large tracts of prairie was completed in 1933 after five years of study. These tracts were distributed over an area of 60,000 square miles, including the eastern one-third of Nebraska, the western one-third of Iowa, and adjacent areas in the four neighboring states (2). This investigation furnished the background for an understanding of the profound changes which have occurred during the several years of the great drought, which first became pronounced in 1934. The response of prairie to the drought of that year has been discussed (3). Likewise, a detailed account of the destruction caused by the drought years of 1934 and 1935 and resultant changes and shifting of populations in both true and mixed prairies have been recorded (1).

Research has been continued from season to season throughout the long period of abnormally low rainfall. Continuous records of climatic and edaphic factors have been obtained, and changes in behavior and structure of vegetation intensively studied. These have been recorded in detail by means of scores of permanent quadrats scattered widely from eastern Nebraska to western Kansas. With successive years of drought further deterioration has occurred. Favorable precipitation during the spring and early summer of 1938 (and during 1937 in certain portions of the area) has brought about important early stages in recovery. Several years with normal or excess precipitation, however, must intervene before the general trends toward readjustment are clearly seen. The purpose of the present paper is to summarize some of the more important changes which have occurred since 1935.

¹ Contribution no. 110 from the Department of Botany, University of Nebraska.

Observations

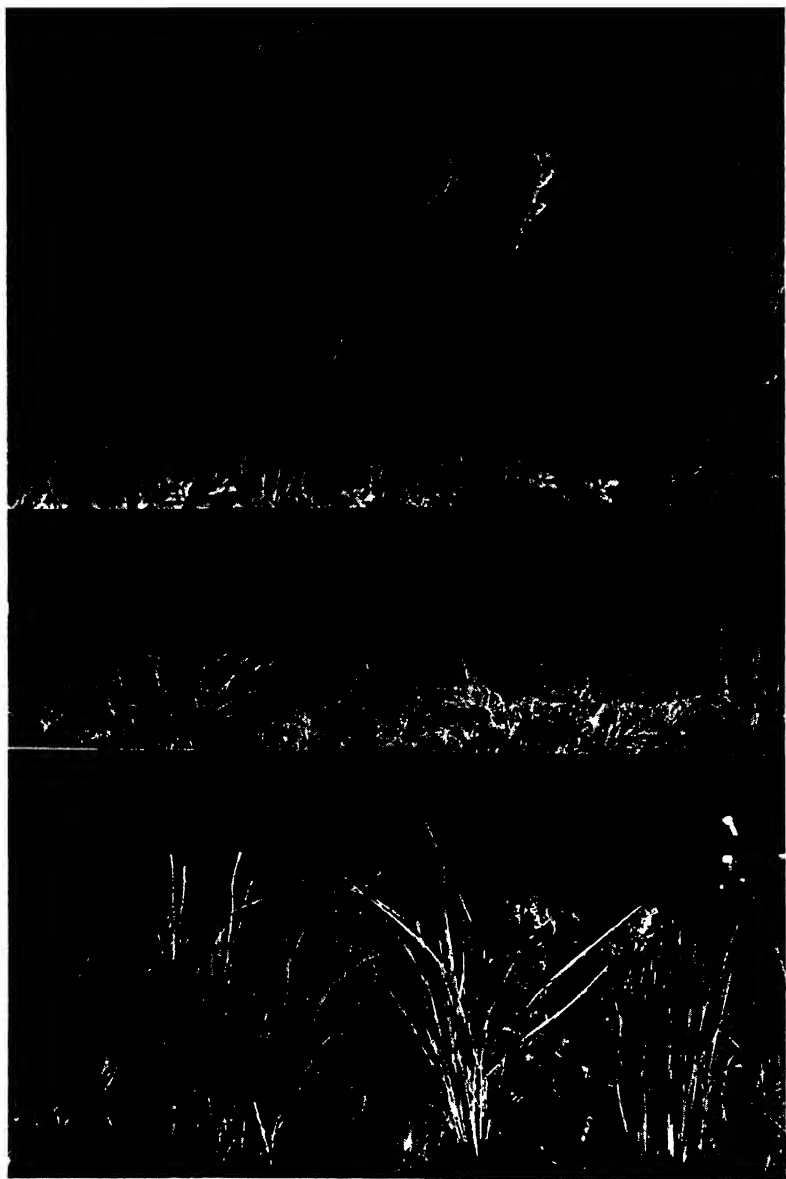
DECREASE IN PERENNIAL GRASSES

Among the most striking effects of the drought is the very great depletion of the formerly most important regional dominant, little bluestem (*Andropogon scoparius*). In numerous prairies not a trace remains; in others small amounts persisted, usually only in the most favorably moist habitats (figs. 1, 2, 3). This loss has been general in true prairie of the southern half of Nebraska. In Iowa this grass has generally persisted but sometimes with a shifting in importance from first to third place, being outranked by *Sporobolus heterolepis* and *Andropogon furcatus*. Since this species alone frequently occupied 50 to more than 70 per cent of the total basal cover on uplands, its death has left open great areas for invasion.

The loss of Indian grass (*Sorghastrum nutans*) has been similar to that of little bluestem. Its nearly complete disappearance is much less noticeable, however, since it formerly constituted only about 2 per cent of the basal cover of vegetation.

The death of Kentucky bluegrass (*Poa pratensis*) has been general in pastures, where it often had nearly complete control, but was somewhat less in prairie because of the shade afforded by mid grasses and forbs. While many prairie areas (especially westward) are practically free of this invader, which normally furnished 5 to 9 per cent of the basal cover on uplands and lowlands respectively, in others it occurs commonly in ravines and at the bases of north slopes. During the favorable season of 1938, these relict patches produced a fine crop of seed and also spread rapidly vegetatively.

That the tall grass, *Andropogon furcatus*, should persist in mixtures where the mid grass, *A. scoparius*, died seems anomalous, but the reason is readily explainable. As the soil moisture became depleted from above downward, little bluestem succumbed when its root system, which is usually about 4 feet deep, had exhausted the available moisture to this depth. The larger and more deeply rooted big bluestem, although much desiccated, persisted. But since the light rainfall of the following years did not add to the scant supply of subsoil moisture, the big bluestem also often succumbed. Its decrease in abundance in 1936-37 has been repeatedly recorded. Thus



FIGS. 1-3.—Upland little-bluestem prairie at Lincoln, Neb., early in August in the same square rod but during different years: Fig. 1, normal stand in 1932 of *Andropogon scoparius* 18 inches tall. Note lower story of *Antennaria campestris* and *Panicum scribnerianum*, and upper one of *Petalostemon candidus* (left), *Amorpha canescens*, and *Liatris scariosa*. Fig. 2, dried bluestem in 1934 only 5 to 6 inches tall and forbs about half normal height. *Solidago glaberrima* (left), *Aster multiflorus*, and *Liatris punctata*. Fig. 3, remaining bluestem (right) in 1938, 12 inches tall; rest has been replaced by *Sporobolus heterolepis* (bunches on left) and *Stipa spartea* (center). Forbs are *Echinacea pallida* (left), *Amorpha canescens*, and *Petalostemon candidus*. Note wide spacing of grasses, complete disappearance of lower layer, and bareness of soil.

over large areas in certain prairies now occupied by needle grass (*Stipa spartea*) or other xeric species, big bluestem has largely vanished from the upland just as little bluestem disappeared during the earlier years of the drought. But this vigorous species, never entirely routed from the uplands, where it formerly constituted as much as 15 per cent of the basal cover, made a remarkable growth during 1938. Having much moist, rich soil with little competition, it reached a height and stem diameter otherwise attained only on lowlands, flowered profusely, and spread rapidly by means of strong rhizomes. It is thus claiming much territory formerly occupied by little bluestem, with which it was so regularly intermixed.

GENERAL DECREASE OF FORBS

The recurrence of years with minimum rainfall has resulted in great decrease of most species of forbs. This pertains to those that root deeply as well as to those of moderate and shallower rooting habit. Some evidence has been obtained that certain species persisting on subsoil moisture at depths below 4 or 5 feet extended their roots even more deeply. But although moisture below the solum was continuous to great depths, repeated sampling showed that it existed only in small amounts. Throughout the drought years rainfall did not augment the supply. Consequently the roots of most individuals came in contact with only definitely limited amounts of deep soil moisture, and many were poorly fitted to profit from water occasionally furnished by rainfall to the surface soil. These conditions, often coupled with desert-like environment aboveground, readily account for the death of increasing numbers of forbs. *Astragalus crassicaarpus*, *Psoralea floribunda*, *Helianthus rigidus*, and *Kuhnia glutinosa* furnish examples of the general situation. Their disappearance was much more gradual and later than that of the shallowly rooted *Fragaria virginiana*, *Antennaria campestris*, and others, which were nearly swept away during the first year. To appreciate fully the paucity of forbs one needs only to examine the prairies eastward across the Missouri River, where they maintained almost their usual abundance. Elsewhere, with exceptions to be noted, losses have been approximated at one-half to two-thirds.

BEHAVIOR OF CERTAIN ANNUAL GRASSES

Following the death of the perennial grasses during the first years of the drought, certain annual species became a veritable scourge. Chief among these were six-weeks fescue (*Festuca octoflora*), chess (*Bromus secalinus* or closely related species), and to a lesser extent little barley (*Hordeum pusillum*). These were nature's shock troops which held the bared soil from eroding until reclaimed by a more stable population. While *Festuca* is still plentiful in most drought-swept true prairies and *Bromus* and *Hordeum* in the western edge, yet none of these are now so continuous in the cover. As will be shown, the major bared or semi-bared areas have been so largely reclaimed by long-lived grasses or by certain native forbs that these annual grasses chiefly fill the smaller interspaces and are not found in large belts or patches as formerly.

DECREASE OF RUDERALS

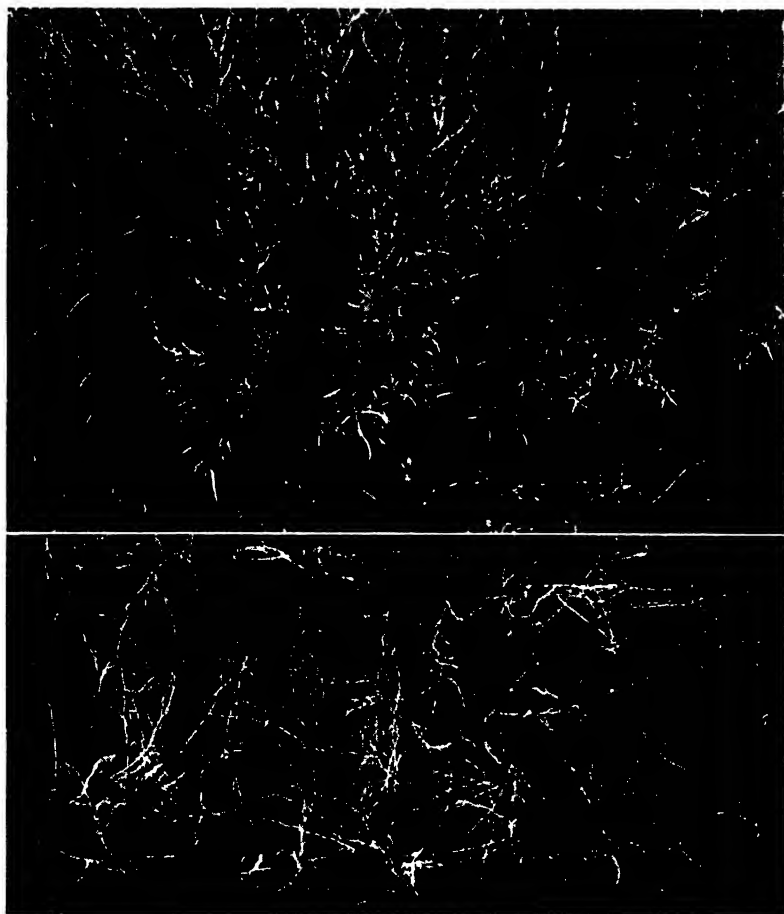
The scourge of pepper grass (*Lepidium virginicum* and *L. apetalum*), so serious in 1936 and 1937 from the Missouri River to the Rocky Mountains, was not in evidence in true prairie during the past year of abundant rainfall. The horseweed (*Leptilon canadense*) likewise persisted in great abundance for only a year or two and has now practically disappeared from the native grassland. More local serious infections of goat's beard (*Tragopogon pratensis*) have now all but vanished. Indeed the absence of ruderals in all the true prairies, except a few where the vegetation was buried under drifting dust, was an outstanding characteristic, especially during 1938.

RESPONSE OF CERTAIN NATIVE FORBS

The chief native forbs that profited by the death of their competitors were *Aster multiflorus*, *Erigeron ramosus*, *Solidago glaberrima*, and (during the first years of drought only) two annuals, *Silene antirrhina* and *Specularia perfoliata*. The last two increased from a ranking of rare or infrequent to one of extreme abundance, populating the drought-bared areas everywhere.

Aster multiflorus, propagating both by seeds and very efficient rhizomes, became so extremely abundant as to ruin many of the prairies or large portions of them for the production of hay (figs. 4,

5). In fact, during 1935-36 it appeared that this forb would completely dominate the prairie. As a result considerable native sod was



FIGS. 4, 5.—Fig. 4, edge of dense patch of *Aster multiflorus* about 2 feet tall. Fig. 5, roots and rhizomes of *Aster* obtained by washing away the soil of one-half square meter area to depth of 10 cm. Photographed Lincoln, Neb., July 25, 1938.

broken because of the seriousness of this pest. Plants with fifty or more woody stems with a spread of tops of 2.5 feet arose from a soil area of one-fourth square foot. Because of the low light intensity (often only 1 to 5 per cent of full sunshine at noonday), seedlings

grew with difficulty, and relict grasses, if present, were greatly attenuated. While *Aster* is still important in occupying many local areas in nearly pure stands, it is plainly waning. In semi-sodded areas a more open type prevails, that is, one to four stems in a place separated from others by rhizomes 2 to 6 inches long. Thus it approaches more nearly its pre-drought condition, where it ranked in general as the third most important upland forb. There was a great decrease in both size and abundance of this forb in 1937, and markedly so in 1938.

The enormous increase of *Erigeron ramosus* in most of the true-prairie areas is one of the outstanding features of the drought period. Always of high rank and great abundance, this typically short-lived biennial thrived in bare areas everywhere. Not only were the plants abundant but also often of greatly increased stature. Profiting by the numerous interspaces between the remaining bunches and mats of grasses, in eastern Nebraska especially, they offered a profusion of blossoms which at a distance appeared as newly fallen snow. *Erigeron* as a forb released from competition and persisting through the drought is an impressive phenomenon. In many of the western true prairies this species scarcely occurs.

Solidago glaberrima has increased greatly in many prairies, and its abundance is revealed especially in July with the yellowing of the inflorescence. One can easily anticipate the degree to which the grasses have been destroyed, that is, the bareness of the soil beneath, by observing the density of the stand of goldenrod.

It is of interest that, in the prairies of Iowa, where the losses by death were much smaller, in general, replacement was not by invading ruderals but by shiftings in the prairie populations. Increase of certain forbs, notably the rhizomatous *Coreopsis palmata* and to a less extent *Aster multiflorus*, was marked. The xeric grass, *Sporobolus heterolepis*, also made gains.

RESPONSE OF CERTAIN FORBS WITH LARGE STORAGE ORGANS

A remarkable increase of certain species, formerly of little importance, in the bared areas has occurred during the last two years. Their wide distribution and dense aggregation were most marked during the moist spring and early summer of 1938. All are char-

acterized by some type of underground storage. In approximate order of abundance, the most important were *Oxalis violacea*, *Senecio plattensis*, *Tradescantia bracteata*, *T. occidentalis*, and *Delphinium virescens*. *Allium mutabile*, *Anemone caroliniana*, and *Erythronium mesochoreum* were also unusually abundant during the drought, although more locally distributed than the preceding. *Oxalis* formed



FIG. 6.—*Oxalis violacea* and *Agropyron smithii* invading drought-bared area. Photographed Crete, Neb., May 22, 1938.

dense patches, usually a few square feet or square yards in area, but such communities were widely distributed in most of the prairies studied (fig. 6). The somewhat fleshy rooted *Senecio*, formerly occurring as scattered individuals or small groups, increased manyfold and was a most conspicuous feature of the vernal aspect. Great groups of the fleshy rooted spiderwort occupied bared areas or were interspersed in the open places between the widely spaced bunch grasses. The thick-rooted larkspur had increased greatly in abundance and in places these usually solitary plants were rather closely

grouped. These species, like the wild onion, wind flower, and dog's tooth violet, had all been favored by a marked decrease in the competition of other prairie components.

INCREASE OF WHEAT GRASS AND CERTAIN OTHER XERIC GRASSES

The great losses sustained by *Andropogon scoparius*, *Poa pratensis*, and other species made possible the enormous increase of more drought-resistant, competing species as well as great invasions by *Agropyron smithii*. The latter phenomenon was by far the most striking feature of the drastic modifications of the prairie.

Extensive earlier surveys showed clearly that western wheat grass, although a dominant of other portions of the true prairie, notably the hardlands of the Dakotas, constituted scarcely any part of this association in the central prairie region. It occurred along roadways, occupied the compacted soil by the sides of trails through the prairie, and was found in abundance locally where a shallow claypan made conditions unfavorable for the growth of the usual dominants. Occasionally a few stems were found about disturbances such as gopher mounds. In native pastures, centers of infestation often occurred on knolls or about gateways where cattle or horses congregated and trampled the soil while stamping to free themselves of flies.

Immediately after 1934, wheat grass became increasingly abundant in both pastures and prairies. During the drought years it has continued spreading over all types of terrain, until today it is often the most important species in many prairies to within 40 miles of the Missouri River. Large circular areas where other species are practically absent indicate the excellent and rapid method of migrating by long, much branched rhizomes. Long narrow belts of wheat grass around the brows of hills indicate where early drought was most severe. Often whole hillsides are covered with pure stands of this grass, and many lowlands are a continuous area of undulating wheat-grass stems. Westward, grasslands hundreds of acres in extent have been three-fourths claimed by this species, and near Lincoln, Nebraska, similar extensive invasions of former bluestem prairies are easily found. Alternates or mixtures occur, but more often the stand is nearly pure. With often a thousand stems per square

meter and a height of 3 to 3.5 feet, this expanse of grassland is an impressive sight (fig. 7). Studies along the invading fronts and the thinning of the stands in some areas with an increase in former dominants give some evidence of a decrease of this rapid spread. But should drought years continue, wheat grass may become even more extensive.



FIG. 7.—Complete disappearance of *Andropogon* and invasion by *Agropyron smithii* (dark) near Jamaica, Neb. Belt of bluestems 2 to 5 rods wide borders postclimax forest along the stream for 80 rods, where it was protected from the hot, dry winds.

Stipa spartea, although at first retarded, has greatly profited by the drought. Its spread has increased enormously and many new centers have been established. This change has been most pronounced in prairies of eastern Nebraska, where invasion by wheat grass has not occurred or has been less extensive. At Nebraska City, for example, a great ridge extends northeastward across a 160-acre prairie. In 1928, only the northern crest of the ridge was dominated by *Stipa*, although it occurred as a species of much less importance than the bluestems along the entire ridge and extended also over the upper slopes. The early death of little bluestem was later followed by great losses of the more deeply rooted big bluestem.

Death of the andropogons, forbs, etc., proceeded gradually down the slopes and the area is now claimed by needle grass. A community of *Stipa* of great extent, with the single dominant furnishing 90 to 98 per cent of the cover of grasses, extends continuously along the crest and spreads far down the slopes. Only near the foot of the hills does one find the bluestems well intact and seriously resisting the down-



FIG. 8.—Open stand of *Stipa spartea* exposing much bare ground. Large portions of these bare areas recently reclaimed by seedlings of this species. Photographed June, 1937.

ward spread of needle grass (fig. 8). Had one not known the composition of the previous cover, the invasion still is clearly evident. Everywhere the cover is open and the soil bare. Only relatively few of the bunches of *Stipa* are large; most are young, small, and widely spaced. Seedlings of needle grass with only a few stems are everywhere, but they are firmly established and only the single stemmed new seedlings may succumb. As many as fifty to sixty small bunches of *Stipa*, one or two large ones, and numerous seedlings are common in a square meter. Similar although less extensive spreading of *Stipa* has been recorded elsewhere.

Prairie dropseed (*Sporobolus heterolepis*) is another xeric species that has spread considerably, especially in eastern Nebraska and western Iowa. In a few prairies it has become the ranking dominant; in many others its importance has greatly increased. *S. asper* has likewise become far more common and sometimes very abundant since the advent of the drought.

There has been a great spreading of the preclimax areas of short grasses. *Buchloe dactyloides*, with the death of its mid-grass competitors, has spread widely along the western fringe of true prairie and *Bouteloua gracilis* wherever it formerly existed, even if in small amounts. The latter was always of greater abundance in uplands and is somewhat more xeric. Increases in area of ten to fifteenfold have been recorded and new outposts have been established in many places. *Carex pennsylvanica*, which is similar in stature to short grasses, has likewise shown marked increases in many prairies and has greatly augmented its importance in pastures.

A grass that formerly occurred sparingly in almost all types of prairie but never attained complete local dominance is side-oats grama (*Bouteloua curtipendula*). During the years of drought it has spread widely until now it is one of the more important of prairie species. It has often persisted where all other grasses succumbed. Its remarkable increase has been due in part to its prolific seeding habit and in part to propagation by rhizomes. In many places it has largely replaced the losses occasioned by the death of little bluestem. In certain southwestern true prairies it has filled in locally bared areas with a pure stand. The bunches are often remarkably large, 6 or more inches in diameter, and the rhizomes 2 to 5 inches long. Elsewhere it often forms half or even more of the prairie cover. Thus the prairie patterns have changed in a most remarkable manner.

REDUCTION OF BASAL COVER, LOSS OF LOWER LAYER AND BARENESS OF SOIL

The great reduction of the basal cover of the prairies generally, except in those relatively small areas where even continued drought left the former prairie carpet intact, is of wide occurrence. It has been a process of eliminating species less fitted to withstand drought, younger plants not yet deeply rooted, and (probably) less vigorous

individuals. From well distributed quadrats, plot estimates, and extensive observations, it has been determined that in general there has been a decrease of basal cover in most drought-stricken areas of 50 to 66 per cent. Small bare areas of one-half to one square foot occur regularly, while larger ones of 1 to 15 square meters with a basal

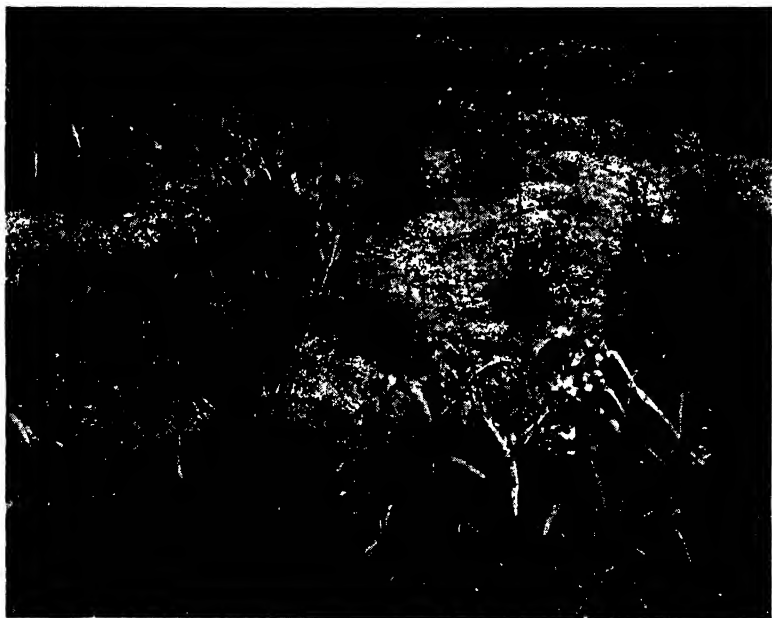


FIG. 9.—Representative bare area caused by continued years of drought. A few of the grasses (*Andropogon*) remained alive; others (*Elymus canadensis*, *Panicum scribnerianum*) are invaders. The forb is *Tradescantia bracteata*. Photographed May 22, 1938.

cover of only 1 per cent are frequent (fig. 9). Where early summer rains were abundant, this wide spacing permitted an excellent growth of foliage. The bunch or sod was filled with stems; the foliage had spread widely. Each species alone produced enough seed to reclaim the entire area. Such a rank growth affords a foliage cover of 70 to 100 per cent. From the same base it may be only 30 to 50 per cent during a dry season. Hence one may be misled and believe that recovery has progressed far beyond the real state. Actually there is room for invasion almost everywhere.

One who knows the prairies of old is constantly impressed with the bareness of the soil. The rank growth of a well developed three-layered vegetation is no more. The ground layer of mosses, lichens, *Antennaria campestris*, low species of *Panicum*, *Viola*, *Astragalus crassicaerpus*, and tufts of *Poa* has practically disappeared. Nor is the soil clothed with the former mulch of fallen leaves, flower parts, stems, etc. Much less of this material has been formed; the lack of moisture and abundance of direct sunshine and wind have caused it to wither and dry or often to blow away, leaving the black soil quite bare. Exceptions are the areas thickly sown by the wind to *Festuca octoflora*, etc., where the stems, lodged, dead, and dried by midsummer, protect the bare soil from sun, wind, and flood.

Both crowns and roots of the dead grasses decayed slowly because of the arid conditions. But after a few years an unusual abundance of dead organic material filled the prairie soil. This promoted the growth of numerous fungi, among which the red stinkhorn (*Simblum sphaerocephalum*) was most abundant and important. During the moist spring and early summer of 1938 this species was found widely and usually thickly distributed in nearly all the prairies. Ten or more individuals per square meter were not uncommon on low ground, and often the air was foul with the nauseating odor produced by this saprophyte.

MODIFICATION OF GRASSLAND PATTERNS

Still another drought feature of the landscape is the sharpness of the alternates marked off as a result of the great destruction, readjustments, and invasions. Here a deep soil area is so located that it escaped the full force of the oven-hot wind; vegetation remained intact. A pace beyond death has struck and *Stipa* or *Sporobolus* has spread to claim the area, or *Koeleria* has repopulated it, or perhaps *Agropyron* is in complete possession. The front and flanks of the mass movement of the latter can clearly be traced usually to within 1 meter, for great distances. Or one may in a step or two pass from pure stands of *Andropogon furcatus* into those of *Bouteloua curtipendula* or even *B. gracilis*. Large circular or irregular areas temporarily dominated by *Aster* or *Erigeron* give way abruptly to a fine growth of *Andropogon furcatus*, or patches of pure *Festuca* alternate with

bare places, as if nature could not in such extremity reseed the whole bare surface. Such sharp transitions are remarkably different from the former intimate intermixtures of climax dominants and the broad ecotones separating the several types. Some of these remain, of course, but in general the change is outstanding.

SIGNS OF RECOVERY

The past year has witnessed an enormous crop of seedlings scattered widely throughout the prairies. Prominent among these have been the grasses. *Bouteloua curtipendula*, *B. gracilis*, *Panicum scribnerianum*, *Andropogon furcatus*, *Koeleria cristata*, and *Stipa spartea* were especially well represented. Seedlings of *Erigeron ramosus*, *Aster multiflorus*, and *Senecio plattensis* ranked highest among the forbs, although a good population of various species of *Psoralea*, *Liatris*, *Kuhnia*, and others was found. Moreover, the few survivors among species of the lower layer are reviving and spreading gradually. Mat plants, bluegrass, and other low-growing forbs and grasses are beginning to reconstruct this layer next to the soil. A few prairies in certain sections favored by two consecutive years of good rainfall are started toward recovery. Although late summer drought destroyed much of the new seedling population, some survived, and with the enormous seed crop of the current year they will repopulate the bared areas if and when good rains prevail.

Summary

1. As a result of the great drought of 1934-37, the most important upland dominant, *Andropogon scoparius*, has suffered great destruction in the southern half of Nebraska.

2. The more deeply rooted *Andropogon furcatus* persisted during the early years of drought but has since suffered heavy losses.

3. *Poa pratensis* was nearly all killed in bluegrass pastures and only relatively small amounts remain in prairies.

4. Most species of forbs, including those very deeply rooted, have gradually succumbed to the continued drought, until they are only one-half to one-third of their former abundance.

5. *Festuca octoflora*, *Bromus secalinus*, and other annual grasses, which became extremely abundant after 1934, have now greatly

decreased in quantity, filling the smaller interspaces rather than occupying large areas.

6. The scourge of the ruderal, *Lepidium virginicum*, so serious in 1936-37, has distinctly disappeared. Other annual weeds were extremely abundant only during one or two seasons.

7. The perennial, rhizomatous *Aster multiflorus* spread so widely into drought-bared areas as to ruin many prairies for production of hay. *Erigeron ramosus* was almost equally widespread.

8. A remarkable increase in abundance of *Oxalis violacea*, *Allium mutabile*, *Tradescantia bracteata*, and other species with thick roots or other storage organs has recently occurred.

9. *Agropyron smithii*, occurring sparsely at the beginning of the drought, has spread so widely as to cover one-half to three-fourths of the area of many former bluestem prairies.

10. Marked increases in territory dominated by *Stipa spartea* and *Sporobolus heterolepis* have appeared. *Bouteloua gracilis* and *Buchloe dactyloides* have greatly increased. *Bouteloua curtipendula*, a dominant, formerly of relatively low rank, has now become one of the most important prairie grasses.

11. Drought has reduced the basal cover in true prairie 50 to 66 per cent. The lower layer of grasses and forbs has been almost destroyed. Grassland types have been much modified.

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INCREASING THE FERTILITY OF *NEUROSPORA* BY SELECTIVE INBREEDING¹

CARL C. LINDEGREN, VIRGINIA BEANFIELD, AND ROBERTA BARBER

(WITH ONE FIGURE)

Haploid ascospores of *Neurospora crassa* germinate to produce either of two mutually exclusive kinds of mycelia, (+) or (−). The sexuality of mycelia can be determined only by mating with a standard tester strain and noting whether or not fertile perithecia (containing ascospores) result. When grown alone neither of these types of mycelia is capable of producing perithecia which contain ascospores, but either a (+) or a (−) mycelium grown alone produces many small sclerotia or bulbils. DODGE (x) has shown that these structures usually cease to grow after attaining a size of about 50 μ , unless they are spermatized, when they develop into perithecia full of ripe ascospores. However, in some unisexual mycelia a considerable number of these tiny bulbils continue their growth until they attain the size of a mature perithecium, thus proving that the growth of the perithecial wall does not depend entirely on bisexual heterokaryosis. None of these unfertilized structures ever contains either ascospores or even rudimentary asci. Asci (even those containing aborted ascospores, 8) are produced only when (+) and (−) strains are mated.

In the standard test for sex, a (−) tester strain is grown together with the tested strain. If perithecia containing ascospores result, the tested strain is labeled (+). However, if the (+) mycelium being tested contains sterility factors which prevent production of ascospores, the mating would fail and the (+) mycelium would be mistaken for a (−) mycelium. It is best therefore to cross an unknown strain with both (+) and (−) tester strains and to withhold judgment unless one cross produces ascospores and the other fails. Often a mating of (+) by (−) produces only sterile perithecia, but in much

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greater abundance than that produced by either unisexual strain grown alone. Occasionally when two mycelia of the same sex are grown together there appear to be more sterile perithecia produced than when either is grown alone.

The fertile inbred strains of *Neurospora crassa*² which have been selected in this laboratory (3, 4, 6, 7) show considerable variation in the perithecia and asci which they produce. When (+) and (−) *fluffy* are mated, numerous perithecia containing an abundance of asci filled with ripe ascospores are produced. Millions of ascospores are ejected from the perithecia on to the walls of the test tube, so that it looks as if it were covered with soot. When perithecia about a month old are crushed, only a few unripe asci can be found in each cluster. However, about one-fifth of the perithecia are empty. The fertility of the *fluffy* strain is so great that when it is mated to a less fertile strain of opposite sex, more ascospores are produced than that strain normally produces if inbred. By contrast to *fluffy*, the *pale* stock produces fewer ascospores. *Pale* by *pale* crosses produce great numbers of perithecia, but most of them are sterile and can be recognized macroscopically by their light brown instead of jet black color. Fertile perithecia have dense black masses of ascospores piled at the ostiole. Sterile perithecia viewed from above show the opening of the ostiole as a white area surrounded by a darker ring. The fertile perithecia of a *pale* by *pale* cross do not contain an abundance of asci filled with ripe ascospores, but when the perithecia are crushed each cluster contains many asci in which unripe or aborted ascospores are found. Microscopic inspection of the walls of the tube is necessary to reveal ejected spores. While both the *fluffy* and *pale* stocks produce many hundreds of perithecia in each test tube, crosses of *even* by *even* often produce only a few dozen perithecia in each test tube. However, these perithecia are usually filled with asci containing ripe viable ascospores.

Maximum fertility apparently depends on one mechanism which operates to develop perithecia from bulbils, and a second mechanism

² In studying cultural differences it is necessary sometimes to keep the cultures out in the laboratory for many weeks, often resulting in infestation with mites. This can be prevented merely by pulling the cotton plug two-thirds out of the tube, dipping it in glycerin, squeezing out the excess, and reinserting it.

which results in the production of ascogenous hyphae. Enlargement of the bulbils occurs when mycelia of opposite sex are grown together, indicating that heterokaryosis of (+) and (−) nuclei stimulates this increase in size. But the fact that growing two mycelia of the same sex together often causes the bulbils to attain the size of mature perithecia indicates that some of the genetical factors involved are independent of (+), (−) heterokaryosis. In the *fluffy* stock the enlarged bulbils are abundant and the ascogenous hyphae are particularly fertile. In a *pale* by *pale* cross the enlarged bulbils are even more abundant, but the ascogenous hyphae produce many aborted ascospores. In *even* by *even* crosses only a few enlarged bulbils are produced, but the ascogenous hyphae are exceptionally fertile.

The standard tester used in this laboratory is highly fertile. It was originally a homokaryotic *tan* strain (no. 1026-5) which reverted to *normal* (3, table 1). After it had been asexually propagated for several years it became heterokaryotic for at least three genetically different strains, *normal*, *peach*, and *clump*, owing to successive mutations (6). Although several mutations occurred during the long period of asexual propagation, the sex genes were unaltered. When this unisexual heterokaryotic mycelium is grown alone on cornmeal agar, it produces an abundance of sterile perithecia, some rather large. The *normal* heterokaryotic unisexual tester strain (1026-5) just described, like the *fluffy* strain, carries factors which compensate for low fertility of other strains to which it may be mated. A cross of *pale* by the tester contains numerous perithecia filled with ripe ascospores; a cross of *even* by the tester likewise contains numerous perithecia filled with ripe ascospores.

This tester strain can be used to diagnose the sexes of ascospores whose mycelia are almost completely sterile *inter se*. In earlier papers the mechanism of crossing over in the sex chromosome has been analyzed by the dissection of the eight spores from each of several hundred hybrid asci, and mycelia were produced by growing these ascospores in tubes by themselves. The progeny of these crosses carried so many sterility factors that numerous matings were necessary between many different pairs of known (+) and (−) mycelia to obtain a cross which produced ascospores for the next generation.

In each successive generation only the relatively more fertile

matings were analyzed. This inbreeding resulted in an increase of fertility in each succeeding generation. The relative increase in fertility of four generations is reported here. All these mycelia were

		(-) MYCELIA																									
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	
(+) MYCELIA	GP 1	A	A	A	A	A	P	A																			
	GP 2	A	A	A	A	A	A	A																			
	GP 3	A	A	A	P	P	A																				
	GP 4	A	A	A	A	A	A	A		A	A	A	A	P	A		P	P		A		A	A	A			
	GP 5	A	A	A	A	A	P	A																			
	GP 6	A	A	A	A	A	A	A																			
	GP 7		P				A	N	N	N	N	A	A	A	P	N	A								A	N	
	GP 8		A				A	A	A	A	A	N	N													N	
	GP 9		P				A	N	N	A	N	P	N													N	
	GP 10		N				N	P	N	N	A	N	N													N	
	GP 11		A	A	A	A	A	A	A	A	A	A	A	A	P	N	N	P		A	P	P	P			N	
	C 12							N	A	A	A	A	A	A												N	
	GP 13														A	P	P	P							N	N	
	GP 14		A				P								P	P	A	P							N	P	
	GP 15		A				P								P	P	P	P							N	P	
	GP 16		A				A								A	P	A	N							N	N	
	GP 17		A				A								P	P	P	P							N	P	
	GP 18		A				A												P	A	P	A	A				
	GP 19		A				A												A	A	A	A	P				
	GP 20		A				A												P	A	A	A	P				
	G 21		A																A	N	A	A	A				
	G 22		A				A												P	A	P	A	A				
	GP 23		N	A	A	A	A			A	A	A	N	A	A	N	A	A	A	A	A	A	A	A	A	N	
	C α														N	N	N	N									

FIG. 1.—Fertility of matings between 23 (+) and 24 (-) mycelia in fourth inbred generation: A, fertile perithecia containing ascospores; P, sterile perithecia; N, no perithecia. Matings blocked in the rectangles were made first, then the mycelia which these first tests showed to be highly fertile were mated with all the others. In this second test 69.4 per cent produced fertile perithecia. Ca was sterile in all matings tested, so its sex is unknown. Second four N matings of Ca not used in calculating percentage fertility.

tested against the highly fertile tester strain to determine their sexes. Then the strains were mated. Since the sexes were known, only (+) and (-) matings were made. In each ascus there are four (+) and four (-) spores. The mycelia of each of the two (-) genotypes were mated with the mycelia of each of the two (+) genotypes in forty-one asci obtained from three successively inbred genera-

tions. In the fourth generation the spores were selected at random and neither the ascus nor the positions in the ascus of the different ascospores were known. Although it had already been established that in each mating the two given strains were respectively (+) and (-), many of the matings failed to produce fertile perithecia.

The first generation was descended from a cross of (+) *gap*, *pale* (+GP) by (-) *crisp* (-C). Six per cent of the matings produced ascospores. In the second generation (+GP \times -C) 30 per cent of

TABLE 1
FERTILITY OF FOUR SUCCESSIVE INBRED GENERATIONS

GENERATION	NUMBER OF MATINGS PRODUCING					
	FERTILE PERITHECIA (A)		STERILE PERITHECIA (P)		NO PERITHECIA (N)	
	No.	%	No.	%	No.	%
First.....	5	5.6	10	11.2	74	83.2
Second.....	15	30.0	8	16.0	27	54.0
Third.....	47	57.2	11	13.4	24	29.5
Fourth (random).....	82	59.0	31	22.3	26	18.7
Fourth (selected).....	50	69.4	13	18.1	9	12.5

the matings produced ascospores. In the third generation (+GC \times -P) and (+GP \times -C) 57 per cent of the matings produced ascospores. In the fourth generation, 59 per cent of the random matings produced ascospores. When the mycelia which this random test showed to be highly fertile were mated with other similarly fertile mycelia of opposite sex, 69 per cent of the matings produced ascospores (fig. 1). The results of these matings were classified as to whether they produced fertile perithecia, only sterile perithecia, or no perithecia (table 1).

The asci used were those reported by LINDEGREN and LINDEGREN (9). One of the commonest types found in dissection is the following: -C, -CP, +G, +GP. In such an ascus the following matings would be made: (1) -C \times +G; (2) -C \times +GP; (3) -CP

× +G; (4) -CP × +GP. The various mycelia originating from the spores of one ascus were mated as follows:

SPORES	GENOTYPE	MATINGS
1.....	-C	1×5
2.....	-C	2×8
3.....	-CP	3×5
4.....	-CP	4×7
5.....	+G	
6.....	+G	
7.....	+GP	
8.....	+GP	

There are five possibilities: (1) none of the matings may produce ascospores; (2) one of the four may produce ascospores; (3) two of the four may produce ascospores; (4) three of the four may produce ascospores; and (5) all of the four may produce ascospores (table 2).

TABLE 2
FERTILITY OF INTRA-ASCUS MATINGS IN THREE SUCCESSIVE GENERATIONS

GENERATION	NUMBER OF MATINGS FROM EACH SET OF FOUR INTRA-ASCUS MATINGS WHICH PRODUCED ASCOSPORES				
	NONE	ONE	TWO	THREE	FOUR
First.....	16	2	1	0	0
Second.....	3	5	3	0	1
Third.....	0	3	2	3	2

The smaller number of crosses analyzed in table 2 as compared with table 1 results from the fact that only in a limited number of asci were all four possible crosses made. Naturally the data in table 2 can be used only if all four crosses have been tried.

SHEAR and DODGE (10) reported that the matings they made between the different species of *Neurospora* produced ascospores, provided the matings were between mycelia of opposite sex. LINDEGREN (1929 unpublished) mated the separate mycelia from one unusual seven-spored ascus of *Neurospora tetrasperma* with six separate

mycelia from an eight-spored ascus of *N. sitophila*, making a total of forty-two matings. Only seven matings produced ascospores; twenty should have done so. Fertility was only 35 per cent. In a second set of matings the same seven mycelia of *N. tetrasperma* were mated to six different mycelia from an eight-spored ascus of *N. crassa*. Only three of the matings produced ascospores; 13.6 per cent fertility. Finally the six *N. crassa* and the six *N. sitophila* mycelia were mated in all combinations. Seven of the matings produced perithecia; eighteen should have; therefore 39 per cent of the matings were fertile.

In all these tests only a single tube was used. That is, if ascospores were not produced in the first mating made, the test is reported N or P. But a single mating with the highly fertile tester is always capable of producing ascospores when opposite sexes are used, so this arbitrary classification is valid. If many subsequent trials are made, however, either a P or even a N cross may produce ascospores in some tubes. Therefore the designation cannot be considered as completely sharp and clear cut. Possibly SHEAR and DODGE studied many different successive matings of the same pair of mycelia in their interspecific crosses or used strains of exceptional fertility.

Summary

1. Mutant strains of *Neurospora crassa* are often extremely infertile.
2. By selecting fertile strains and inbreeding, it is possible to increase fertility.
3. The development of the perithecium and that of the ascogenous hyphae seem to be controlled by independent mechanisms.

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PHLOEM DEVELOPMENT AND FLOWERING¹

B. ESTHER STRUCKMEYER AND R. H. ROBERTS

(WITH EIGHTEEN FIGURES)

Introduction

WILTON and ROBERTS (2, 3) reported that the anatomy of the flowering plant is different from that of the non-flowering plant. They found a similarity in the anatomical structure of all flowering plants examined although a wide range of environmental treatments was needed to secure flowering. Observations with respect to the details of the anatomy of the phloem of flowering and non-flowering plants are presented in this paper.

Material and methods

Most of the plants used in this investigation were grown in the greenhouses and the gardens of the horticultural department of the University of Wisconsin. The plants in the greenhouse were grown under several light and temperature conditions (1). The responses to temperature and light were noted and samples were taken for an anatomical study of the stems. The non-flowering and flowering plants were planted at the same time and so were of the same age when sampled.

Samples of flowering stems were taken at the second internode below the inflorescence, and non-flowering stems were sampled at the third well developed internode below the tip of the shoot. To avoid the transition stages occurring between flowering and non-flowering stems, considerable care was taken to insure that samples of strictly vegetative and flowering plants were secured.

As a further check on the relation of the anatomical condition to flowering, several species of plants were pruned to induce new shoot growth from axillary buds. Some of these new shoots produced flowers, while others remained vegetative. Samples were taken of the internode below the node from which the new branches arose.

¹ Published with the approval of the Director of the Agricultural Experiment Station.

In order to obtain samples of non-blossoming stems, it was necessary to defoliate or place in the shade those plants which have a persistent tendency to flower. A number of plants such as tobacco, mustard, *Cleome*, and *Tropaeolum* which had been pruned were placed in the shade to provide an environment in which the regenerated shoots would not blossom.

The samples of the stems were fixed in formalin-acetic-alcohol, and normal butyl-alcohol was employed as the dehydrating agent. Cross sections of the paraffin imbedded samples were cut 12-18 μ in thickness. Safranin and light green proved satisfactory for staining these sections. Longitudinal sections were prepared to determine the presence of callose.² These sections were stained with China blue and iodine-potassium-iodide.

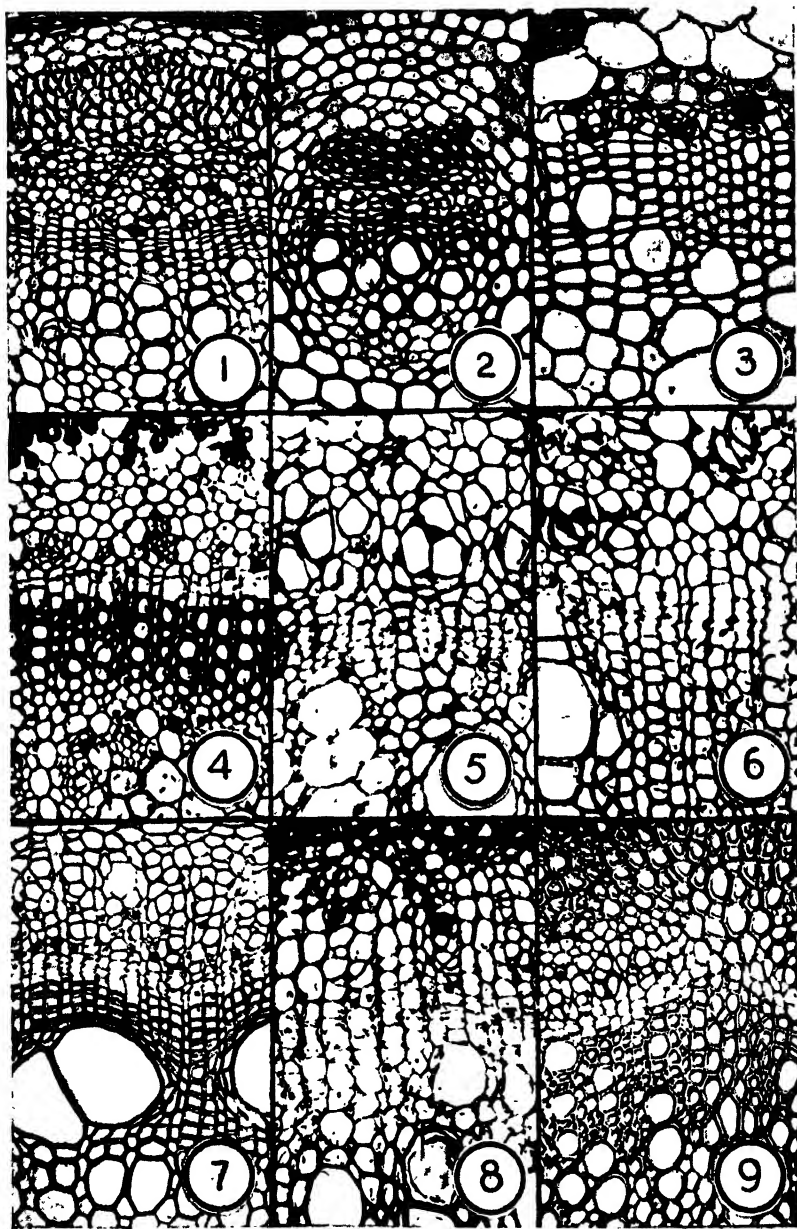
Observations and discussion

From the study of eighty-five species and varieties of plants it was found that the structural details of the phloem of the flowering and non-flowering plants are different. The phloem of the flowering stem differs from that of the vegetative stem in that there is a less amount of this tissue, the sieve tubes and companion cells are smaller, the walls of the cells are thicker, some of the cells in some species are crushed, parenchyma is formed adjacent to the cambial region, and more callose is present in the sieve tubes.

These details of the phloem are present in an unequal degree in flowering stems of different species. In each case, however, three or more of these characters are present which distinguish the phloem of the flowering from that of non-flowering stems.

A significant feature of the phloem of the flowering stems is the limited formation of sieve tubes and companion cells following the reduced cambial activity which precedes blossoming. *Ageratum*, *Zinnia*, *Dianthus*, *Hedalgo wercklei* (figs. 1, 2), and hemp illustrate

² The misuse of the term callus for callose has frequently occurred in literature. According to Webster, callus is the soft parenchymatous tissue which forms over any wounded or cut surface of a stem. When the wound occurs on stems exposed to the air, the callus soon forms a cork cambium. Callose, on the other hand, is a deposition on the cell wall, especially in sieve tubes, differing from cellulose in that it is insoluble in Schweitzer's reagent and soluble in soda solution. In addition it is easily colored by aniline blue and rosolic acid.



FIGS. 1-9.—Portions of transverse sections of stems: Figs. 1, 2, *Hedaldo wercklei* (1, non-flowering; 2, flowering). Figs. 3, 4, *Fagopyrum esculentum* and *Vinca major* (both flowering plants). Figs. 5, 6, *Ricinus communis* (5, non-flowering; 6, flowering). Fig. 7, *Ipomoea batatas* (non-flowering). Figs. 8, 9, *Tagetes erecta* (8, non-flowering; 9, flowering).

this condition. Attention was given to those plants which are difficult to keep from flowering and also to those which flower only rarely. *Fagopyrum esculentum* (fig. 3) and *Begonia semperflorens* flower more or less continuously under greenhouse conditions. It was observed that the phloem cells in these plants are fewer in number than in those plants which rarely flower. *Vinca major* (fig. 4), for example, flowers only very occasionally, and the cells of the phloem even in the flowering stems of these plants are relatively numerous. Also a plant such as the sweet potato (fig. 7), which could not be induced to flower under our cultural treatments, showed a large amount of phloem.

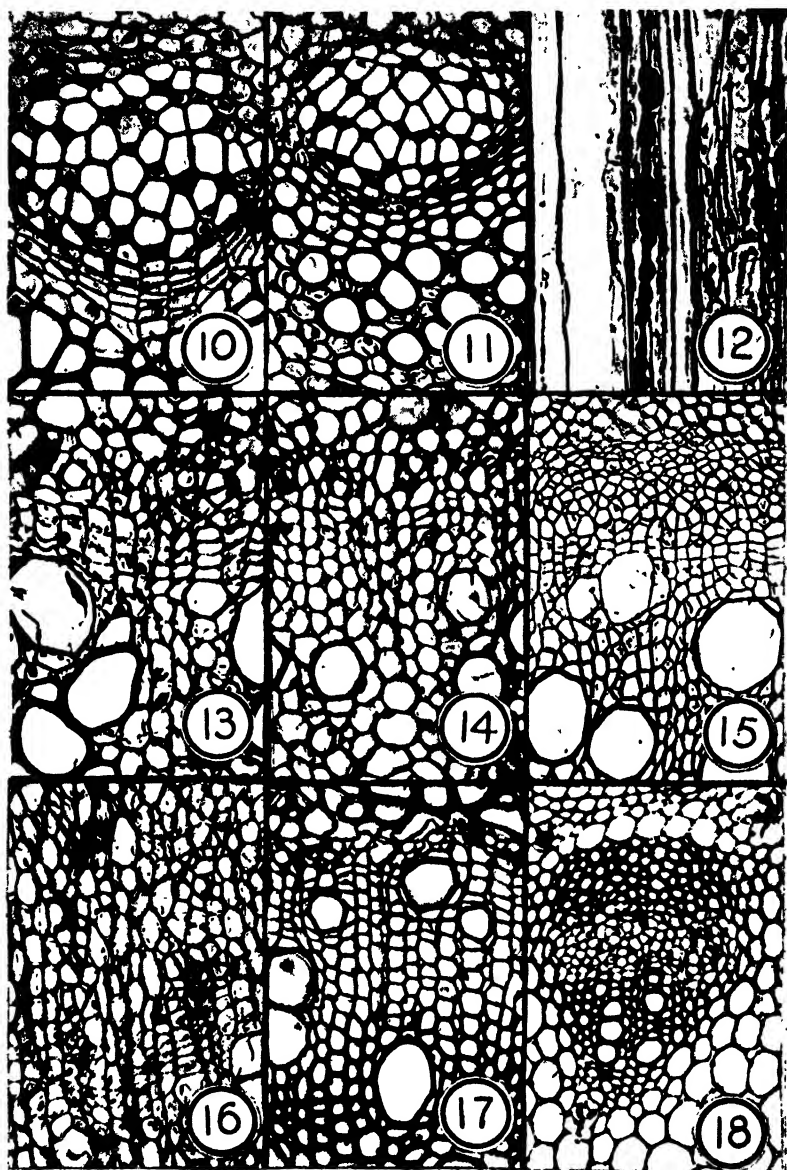
In the castor bean (figs. 5, 6), potato, marigold (figs. 8, 9), *Delphinium*, and a number of other plants the sieve tubes and companion cells are smaller in the flowering than in the non-flowering stem. In the majority of cases the walls of the cells of the phloem appear to be more thickened in the stems of the flowering plants.

Another characteristic of the stems of some flowering plants is the crushing of some of the phloem cells. A plant such as *Delphinium* (figs. 10, 11) shows sieve tubes and companion cells that have collapsed. Flowering stems presenting a similar condition are *Tropaeolum*, snapdragon, *Cleome*, and *Clematis*.

As the cambium ceases to be meristematic, phloem parenchyma rather than sieve tubes and companion cells is differentiated. This is well illustrated in stems of marigold, geranium, castor bean, and potato (figs. 13, 14).

In those plants in which callose was observed, as in geranium, alfalfa, and hemp (fig. 12), it is evident that the amount of callose on the sieve plates and sieve fields is greater in the stems of flowering plants than in the stems of non-flowering plants. The flowering stem of the ornamental lemon has a large amount of phloem tissue, but many more of these phloem cells have callose than have the cells in the non-flowering stem.

In a regenerating stem such as that of marigold, which has flowered but later became vegetative following pruning, two distinct regions of sieve tubes and companion cells are present (fig. 16). That is, the first group of sieve tubes and companion cells was formed before the plant blossomed, and during the flowering period



FIGS. 10-18.—Portions of sections of stems: Figs. 10, 11, *Delphinium ajacis* (10, non-flowering; 11, flowering). Fig. 12, *Cannabis sativa* (longitudinal section of flowering plant). Figs. 13, 14, *Solanum tuberosum* (13, non-flowering; 14, flowering). Figs. 15, 18, *Cosmos sulphureus* (15, non-flowering; 18, disbudded). Fig. 16, *Tagetes erecta* (flowered and later became vegetative following pruning). Fig. 17, *Fagopyrum esculentum* (below newly regenerated shoot which flowered immediately without period of vegetative growth).

phloem parenchyma was formed from the cambium. As the plant again became vegetative, the cambium was active and sieve tubes and cambium cells were again differentiated. In contrast to this condition, if the newly regenerating shoots immediately flower without a period of vegetative growth, as in *Fagopyrum esculentum* (fig. 17), there is practically no new phloem present. In such cases the new ring of tissue consists almost entirely of xylem cells.

Studies reported here are of samples of plants which are definitely vegetative or in advanced stages of flowering. One might ask if the anatomy which is characteristic of flowering stems is a result of flowering. It has been found from repeatedly disbudding plants such as *Cosmos* that the anatomy which is typical of flowering plants was present although they were prevented from flowering by disbudding (figs. 15, 18).

These observations raise the question: Do these details of the phloem suggest that an interrupted transport of elaborated materials causes blossoming in much the same way that girdling commonly results in flower production? The answer to this question may be partly solved by studies which are being made of samples collected during the period of transition from the non-blossoming to the blossom state. Sufficient progress has been made in the inspection of this material to permit of the preliminary statement that changes in the details of the anatomy of the phloem have been observed within a few days after the start of a change in environmental treatment, as short or long day, and as early as the appearance of blossom primordia.

Summary

From observations of the phloem of stems of a large number of plants, some structural differences between flowering and non-flowering plants were noted. There is a greater number of sieve tubes and companion cells in the non-flowering than in the flowering stem, and these cells are generally also larger in the vegetative stems. Some of the phloem cells in the flowering stems of many plants are crushed. The cambium in the flowering stem becomes less active and phloem parenchyma instead of sieve tubes and companion cells is formed. There is more callose in the phloem cells of the flowering

than in the non-flowering stem. These characteristics have been observed at the same time as the appearance of blossom primordia in several species.

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NITROGEN NUTRITION IN RELATION TO PHOTOPERIODISM IN *XANTHIUM* *PENNSYLVANICUM*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 499

EDITH K. NEIDLE

(WITH ONE FIGURE)

Introduction

Many attempts have been made to correlate the effects of nutrient supply and the effects resulting when plants are subjected to various photoperiods. In general these attempts have shown that most plants which bloom at a short photoperiod cannot be induced to flower if grown continuously on long photoperiod, nor can plants which flower only on long photoperiod be induced to flower on short photoperiod, despite wide variations in the plane of nitrogen supply. MURNEEK (2) has recently reviewed the subject critically and added many experimental data.

Despite the fact that the induction of flowering in some plants seems to be more or less independent of the nitrogen supply in the nutrient medium, some of the other developmental phases of plants subjected to various photoperiods are definitely correlated with it. HAMNER (1) has pointed out that under the term flowering and fruiting condition, several developmental phases are included, such as: (1) the induction of the flowering condition, (2) the initiation of floral primordia, (3) the development of the floral primordia into mature flowers, (4) the development of fruits, (5) gametic union, and (6) the development of seeds. These phases are not all alike in the manner or extent to which they are maintained in relation to environmental factors, and should therefore be considered both independently and as they may be related to one another. It is with some of these points that this article deals.

The work was undertaken to determine what effect various planes of nitrogen supply may have upon the development of flowers and fruits in *Xanthium*, provided the plants are given a sufficient number of short photoperiods to bring about the induction of flowering and

the initiation of floral primordia. In general the plants were grown on long photoperiods of approximately 17.5 hours until they were about 10 inches tall. At this time all the plants were given an induction period of seven 8-hour photoperiods and the same number of 16-hour dark periods, with the exception of a few plants which were continued on long photoperiods to serve as controls. At the end of the induction period all plants subjected to the 8-hour photoperiod had initiated flower primordia. Half of them were then returned to long photoperiod and half continued on short photoperiod. The nitrogen supply to the plants was varied either by giving them a nutrient solution containing an abundance of nitrate nitrogen or one containing none. Two separate experiments were carried out and the results were approximately the same. For this reason only the results of one of them, from August 20 to January 19, are given here in detail.

Material and methods

Mature fruits of *Xanthium pennsylvanicum* were collected in the autumn of 1936 and stored in a dry place at room temperature. In August, 1937, the seeds were carefully removed from the burs and scratched slightly so as to rupture the seed coats. On August 20, six seeds were planted in each of sixty 2-gallon glazed crocks containing a medium grade of quartz sand. They were watered with distilled water for the first 7 days and thereafter a nutrient solution was supplied every third day. When germination had occurred, on August 25, 200 watt Mazda lamps, placed about 3 feet above the plants, were turned on each evening at 6:00 P.M. and off at midnight, giving the plants a total daily photoperiod of between 17 and 18 hours. During the very short days of the winter months the lamps were turned on at 4:30 P.M.

The following nutrient solutions were used:

PLUS N SOLUTION		MINUS N SOLUTION	
NaNO ₃	638 p.p.m.	KH ₂ PO ₄	212 p.p.m.
KH ₂ PO ₄	212	MgSO ₄	695
MgSO ₄	695	CaCl ₂	339
CaCl ₂	339	ZnCl ₂	1
ZnCl ₂	1	MnSO ₄	1
MnSO ₄	1	FeSO ₄	1
FeSO ₄	1	Na ₂ B ₄ O ₇ ·10 H ₂ O ...	1
Na ₂ B ₄ O ₇ ·10 H ₂ O ...	1		

The first solution was supplied to all the plants until September 13. The plants were then approximately 6 inches tall and had two or three fully expanded leaves. On this date a division into two groups was made, one group of thirty pots was continued on the +nitrogen solution and the other group of thirty on the -nitrogen solution. On September 20 those plants which were receiving the -nitrogen solution were slightly smaller and somewhat more yellowish than the other plants; they showed no nitrates present on the basis of the di-phenylamine test.

On this date the number of plants was reduced to four per pot. Examination under the dissecting microscope showed no slightest indications of flower primordia. Two pots of plants from each treatment were so continued and subjected to long photoperiod. These served as controls. The remaining plants were transferred to a short photoperiod, to which they were subjected for 7 days. At the end of this induction period dissections showed that all the plants so treated had differentiated floral primordia.

At this time, therefore, four lots of plants were available for further experimentation: (1) those which had been grown continuously on long photoperiod and supplied with the + nitrogen solution; (2) those which had been grown continuously on the long photoperiod but which had been supplied with the -nitrogen solution subsequent to September 13; (3) those which had been given an induction period of seven short photoperiods and which had been continuously supplied with the +nitrogen solution; and (4) those which had been given an induction period but which had been supplied with the -nitrogen solution subsequent to September 13. Lots 1 and 2 were continued on the same treatment as previously given. Half of lot 3 and of lot 4 were continued at the short photoperiod and the other half of each lot returned to long photoperiod. A further subdivision was then made so that of the plants on long photoperiod half were placed on the +nitrogen solution and half on the -nitrogen solution. Thus there were ten groups of experimental plants. The treatments which the various groups of plants received are listed in figure 1. Those groups which were exposed to an induction period, and which developed flowers as a result of this treatment, are listed as groups II to IX. Thus certain of the plants were allowed to develop fruits on a short photoperiod and others on a long photoperiod.

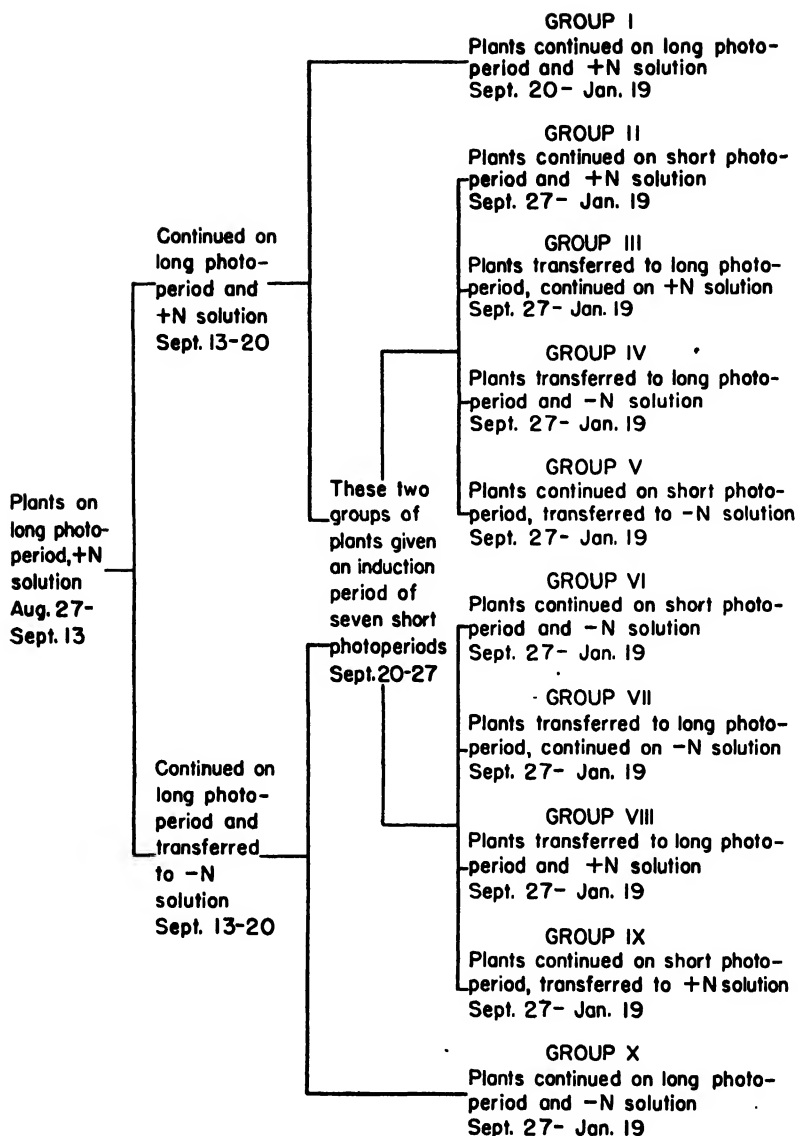


FIG. 1.—Diagram showing groups of plants used throughout the experiment

Results

In the tabulation and discussion of results the various lots of plants are referred to according to the listing in figure 1. Observations were made on the plants daily. The first plants to shed pollen did so on October 14, 17 days after the end of the 7-day induction period.

DEVELOPMENT OF STAMINATE INFLORESCENCES.—The rate of development of the staminate heads, the number produced, and the fertility of the pollen were all affected by both the length of photo-

TABLE 1
NUMBER OF PLANTS OF THE VARIOUS GROUPS SHEDDING
POLLEN ON GIVEN DATES

GROUP	NO. OF PLANTS SHEDDING POLLEN				NO. OF PLANTS
	OCT. 14	OCT. 15	OCT. 19	OCT. 20	
II.....	7	10	25	25	27
III.....	0	0	5	19	19
IV.....	0	0	2	22	22
V.....	7	8	15	15	24
VI.....	0	1	13	13	25
VII.....	0	0	0	21	21
VIII.....	0	0	4	11	11
IX.....	1	3	20	20	22

period and the nitrogen supply after the induction period. The final results of the dates of pollen shedding by the plants of the various groups, the number of plants producing no staminate flowers, and the total number of staminate heads produced are shown in tables 1 and 2. The percentage of abortive pollen grains in the various groups is shown in table 3.

Those plants which were transferred to long photoperiod after the induction period developed flowers more slowly than those which were continued on short photoperiod. Although these differences are represented by a few days only, still these few days represent a considerable portion of the period of development, as for example, in the case of those from which pollen was shed 17 days after the end of the induction period. While those plants returned to long photo-

period developed flowers more slowly, nevertheless the staminate heads finally produced were larger and many more were produced than on those plants continued on short photoperiod. Every plant that was placed on long photoperiod subsequent to the induction

TABLE 2
PRODUCTION OF HEADS IN PLANTS OF
SHORT PHOTOPERIOD

GROUP	NO. NOT PRO- DUCING STAMI- NATE HEADS	TOTAL NO. OF STAMINATE HEADS PRODUCED
II.....	2	80
V.....	9	34
VI.....	12	18
IX.....	2	54

TABLE 3
NUMBER OF ABORTIVE POLLEN GRAINS PER 100

GROUP	OCT. 28	OCT. 30	OCT. 30 (2ND COUNT)	OCT. 31	NOV. 1	NOV. 3	AVERAGE
II.....	84	59	80	70	64	0*	71.4
III.....	1	8	0†	4	9	1	4.6
IV.....	10	5	0†	8	3	9	7.0
V.....	38	0*	0*	9	18	26	22.5
VI.....	23	26	20	14	25	5	20.3
VII.....	79	2	0†	11	10	15	23.0
VIII.....	15	6	0†	4	15	10	10.0
IX.....	46	20	30	46	60	57	40.4

* Count was not possible because the whole flower cluster had dead anthers.

† No second count made.

period produced numerous staminate heads and these were produced over a long period of time, new ones developing several weeks after the first one had shed its pollen. On the other hand, the plants growing on short photoperiod produced relatively few staminate heads, some of them producing none, and those that were produced soon shed their pollen and no new ones developed. The amount of pollen abortion also was much greater in the plants continued on short photoperiod (table 3). These data are the result of frequent

observations of pollen grains prepared by the aceto-carminic smear technique and examined with the microscope.

Those plants receiving a low supply of nitrogen and returned to long photoperiod after the induction period more closely resembled the plants on short photoperiod treatment than did the plants which were continued on a high nitrogen supply and returned to long photoperiod. In contrast to this, those plants on short photoperiod with a high nitrogen supply more closely resembled the plants on long photoperiod (tables 1-4).

TABLE 4
SIZE OF ANTHERS OF VARIOUS GROUPS GROWN ON
DIFFERENT TREATMENTS

GROUP	MEASUREMENTS IN MM.			
	LENGTH (BASE OF POLLEN AREA TO TIP)	WIDTH	LENGTH OF POLLEN BEARING AREA	WIDTH OF POLLEN BEARING AREA
II.....	0.891	0.286	0.542	0.116
III.....	1.085	0.333	0.790	0.115
VI.....	0.910	0.298	0.581	0.146
VII.....	0.910	0.340	0.705	0.186

DEVELOPMENT OF PISTILLATE HEADS.—The effect of nitrogen supply upon the development of pistillate flowers was somewhat similar to that upon staminate flowers. In every case the more abundant the supply of nitrogen the greater the number of carpellate flowers. This was true under both the long and the short photoperiod conditions. Plants on short photoperiod developed pistillate flowers much more rapidly and were receptive to pollen 2 weeks before any flowers from the plants on long photoperiod. Data for the carpellate flowers counted on October 21 are given in table 5.

Those plants which were returned to long photoperiod after the induction period produced relatively few pistillate flowers and relatively few burs (table 6). The total number of burs produced by the plants on short photoperiod was far greater than the total number produced by the plants on long photoperiod. Many of the fruits

from plants on short photoperiod contained no seeds. In group VIII, although 711 burs were produced, only 132 contained seeds, and many burs developed parthenocarpically. Perhaps one of the reasons for so many of the short photoperiod fruits developing without seeds might be correlated with the fact that a number of carpellate flowers were receptive for pollination before any pollen was available. That

TABLE 5
NUMBER OF PISTILLATE FLOWERS PRODUCED IN
INDIVIDUAL PLANTS BY OCTOBER 21*

GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII	GROUP VIII	GROUP IX
24	5	3	15	17	1	0	24
23	6	2	25	16	0	3	15
24	4	3	13	6	1	3	12
21	5	3	15	12	0	2	24
27	0	5	20	11	3	5	13
15	5	2	11	7	3	1	29
18	6	3	15	8	1	0	15
27	0	4	21	9	3	4	16
16	5	3	8	5	2	0	13
33	2	3	23	6	1	0	20
19	4	4	12	6	1	1	27
20	0	28	7	0	5	25
28	7	14	9	4	2
33	3	22	17	5	3
36	7	17	1	0
14	7	20	4
AVERAGE PER PLANT							
21.4	4.1	3.1	15.2	10.8	1.7	2.0	16.4

* After this date the plants growing on long photoperiod (groups III, IV, VII, VIII) produced more flowers.

is, the pistillate flowers on the short photoperiod treatment developed more rapidly than the staminate flowers, and the styles and stigmas withered away before pollination had taken place.

The weight of the burs produced on long photoperiod was approximately twice as great as those on the short photoperiod (tables 7, 8). The weight of the seeds of the various groups was also greatly affected.

In every case the plants grown on short photoperiod produced only one crop of burs. Shortly after maturation of these, the plants

under this treatment died. Those plants on the long photoperiod having little nitrogen available (groups IV and VII) were comparable in this respect with short photoperiod plants in that they produced only one crop of burs. While an abundant supply of nitrogen in its effect upon the number of burs developed is somewhat similar to the effect of long photoperiod, in its effect upon the weight of seeds it is in direct contrast to the effect of length of photoperiod. Thus the burs of the plants on the +nitrogen treatment were always lighter than the burs on the -nitrogen treatment, whereas the individual seeds of the former were always heavier than those of the latter.

TABLE 6
NUMBER OF BURS PRODUCED BY GROUPS OF PLANTS
OF THE VARIOUS TREATMENTS

GROUP	NUMBER OF BURS					
	TOTAL	AVERAGE PER PLANT	No. WITH SEED	AV. WITH SEED PER PLANT	No. WITH- OUT SEED	No. IN 2ND CROP
II.....	521	21.7	181	7.1	340	0
III.....	201	10.5	200	10.5	1	25
IV.....	158	7.0	156	7.0	2	0
V.....	335	15.5	165	7.1	170	0
VI.....	258	10.2	47	1.9	211	0
VII.....	103	5.0	99	4.7	4	0
VIII.....	96	8.7	96	8.7	0	5
IX.....	711	32.3	132	6.0	579	0

The length of the photoperiod had more effect than the nitrogen supply in determining the total heights of the plants, as shown in table 8. The plants grown on long photoperiod were in general about 5 inches taller than the plants grown on short photoperiod. The plants returned to long photoperiod did not die after producing one crop of fruit, but continued growing slowly for a long time. Certain plants were placed under continuous illumination on December 15 and were continued on this treatment for more than 7 months. During this entire period these plants continued to grow and produce additional flowers and fruits. Many of the bracts in the staminate inflorescence somewhat resembled green foliage leaves. Certain of the staminate flowers possessed ten or more stamens in a single

flower. In certain cases, after a staminate head had been partially formed, the apical internodes of the axis of the staminate inflorescence continued elongation, producing one or more long internodes,

TABLE 7
NUMBER AND WEIGHT OF BURS PRODUCED BY
PLANTS OF THE VARIOUS GROUPS

GROUP	NO. OF BURS	WEIGHT OF BURS (GM.)	AVERAGE WEIGHT OF BURS (GM)
II.	198	21.0	0.106
III			
1st crop	175	35.9	.205
2nd crop	25	4.7	.188
IV	156	33.5	.214
V.	150	17.0	.113
VI	49	6.5	.132
VII	99	21.5	.217
VIII			
1st crop	144	31.2	.216
2nd crop	6	1.2	.200
IX.	120	15.5	0.129

TABLE 8
WEIGHT OF SEEDS PRODUCED BY PLANTS OF THE VARIOUS
TREATMENTS AND THEIR AVERAGE HEIGHT

GROUP	TREATMENT	WEIGHT OF 10 SEEDS (GM.)	AVERAGE HEIGHT (INCHES)
II	Short photoperiod		
V.	+NO ₃	0.790	13 $\frac{3}{4}$
IX.	+ and -NO ₃	.683	12
VI	- and +NO ₃	.780	13 $\frac{1}{2}$
	-NO ₃	.723	11 $\frac{1}{2}$
III.	Long photoperiod		
IV	+NO ₃	.912	19 $\frac{1}{2}$
VIII.	+ and -NO ₃	.690	17 $\frac{1}{2}$
VII.	- and +NO ₃	.905	18
	-NO ₃	0.883	14 $\frac{3}{4}$

and then a new staminate head would be formed. As a result of the intermittent vegetative growth and flowering, some of the plants on long photoperiod increased in height manyfold during the months following the 7-day induction period. The vegetative growth of the

plants on long photoperiod was made only by those plants which received an abundant supply of nitrogen. Those plants which received a limited supply, after the induction period, produced one crop of fruit and soon died. No matter how abundant the supply of nitrogen to the plants on short photoperiod, they did not grow vegetatively to any marked degree, whereas the nitrogen supply to the plants returned to long photoperiod had a very definite effect.

In comparing plants which had been subjected to an induction period with plants which had not been so treated but which had been grown continuously on long photoperiod, and consequently were vegetative, certain differences were observed. The controls growing on long photoperiod and with an abundant supply of nitrogen produced a great amount of vegetative growth over a period of several months. These plants branched very little, but attained a height of more than 5 feet. The plants on long photoperiod, which had received an induction period of seven short photoperiods and which also had received an abundant supply of nitrogen, did not produce as much growth but branched more profusely. The control plants continued on long photoperiod but transferred to a minus solution on September 13 produced relatively little growth in height. In comparing these plants with those grown on a —nitrogen solution and a long photoperiod and which had received an induction period, the control plants were smaller and had less dry weight. Microscopic examination of the stems showed that the control plants were smaller in diameter and contained many starch grains and calcium oxalate crystals. The plants of group VI, on the other hand, were larger in diameter and contained no starch grains or calcium oxalate crystals.

Summary

1. Plants of *Xanthium pennsylvanicum* were grown in sand cultures containing either an abundance of nitrogen or very little. They were first grown on long photoperiod. Then all were given an induction period of seven short photoperiods and then one-half were returned to long photoperiod, the other half continuing on short. The plants continued on short photoperiod and receiving an abundant supply of nitrogen bloomed first; those of the same type at a low supply of nitrogen bloomed next; those on long photoperiod and

high nitrogen supply were the third to bloom; those on long photoperiod and low nitrogen were the last to bloom.

2. Of the short day plants, those receiving an abundant supply of nitrogen produced the greatest number of staminate heads. All the plants on long photoperiod produced more staminate flower clusters than the plants on short photoperiod. The percentage of abortive pollen grains was greatest on the plants on short photoperiod. In the latter a low plane of nitrogen supply resulted in the greatest number of defective pollen grains, and in the plants on long photoperiod a high supply of nitrogen resulted in the same effect.

3. There were more fruits per average plant which received a short photoperiod than a long one. Whether the photoperiod was long or short, those given an abundant supply of nitrogen had many more burs than those given a low supply. More seeds were found on plants on long photoperiod because many of them on short photoperiod had empty burs, many of the latter developing parthenocarpically.

4. Plants grown on a long photoperiod following an induction period with a low supply of nitrogen bore fruits and were larger than control plants grown on a similar long photoperiod with no induction period but with a similar low nitrogen supply. The latter bore no fruits.

This investigation was carried out under the direction of Dr. K. C. HAMNER, Department of Botany, University of Chicago.

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STUDIES OF PROTOPLASMIC STRUCTURE IN SPIROGYRA. IV. EFFECTS OF TEMPERATURE ON PROTOPLASMIC ELASTICITY¹

HENRY T. NORTHEN

(WITH ONE FIGURE)

Introduction

Because the effect of temperature is not entirely an effect on the actual chemical processes, as evidenced by inconstancies of Q_{10} 's and lowerings of optimum temperatures with increases of exposure, it is conceivable that temperature alters the structure of protoplasm and that such alterations influence the character and rate of various processes.

The data presented in this paper indicate that as the temperature is raised from 1° to 38° C., the elasticity of the protoplasm in cells of *Spirogyra* decreases. Presumably changes in elasticity involve alterations in protoplasmic structure. The possible nature of such alterations is discussed and an attempt has been made to correlate alterations in structure with some biological phenomena.

The elasticity of the protoplasm was considered decreased if increases in temperature lowered the value of c_0 in the equation $V = k(c - c_0)$, which approximately governs the rate of chloroplastic movement in cells of *Spirogyra* in response to centrifugal accelerations (7). In the equation, V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration used, and c_0 the initial starting acceleration at which or below which the chloroplasts will not move regardless of how long the cells are centrifuged.

Method

Filaments of *Spirogyra* were laid between two strips of moist cotton, and these cotton wads were then placed in centrifuge tubes.

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The tubes were immersed for the desired time in a constant temperature bath (durations of immersion and temperatures used are recorded in table 1). Next the tubes were removed and the filaments

TABLE 1
EFFECT OF TEMPERATURE ON VALUE OF c_0 IN EQUATION $V = k(c - c_0)$

EXPERIMENT NO.	TEMPERATURE (°C.) AT WHICH FILAMENTS WERE MAINTAINED	MINUTES IMMERSED	CENTRIFUGAL ACCELERATION \times GRAVITY	MINUTES CENTRIFUGED	PERCENTAGES OF FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED
1	$\left\{ \begin{array}{l} 1.0 \\ 5.0 \\ 6.5 \\ 8.5 \\ 11.5 \end{array} \right.$	6	1062.0	0.25	$\left\{ \begin{array}{l} 1 \\ 25 \\ 37 \\ 35 \\ 61 \end{array} \right.$
2	$\left\{ \begin{array}{l} 1.0 \\ 5.0 \\ 6.5 \\ 8.5 \\ 11.5 \\ 15.0 \\ 19.0 \\ 23.0 \end{array} \right.$	6	680.0	0.50	$\left\{ \begin{array}{l} 0 \\ 4 \\ 11 \\ 22 \\ 35 \\ 61 \\ 65 \\ 93 \end{array} \right.$
3	$\left\{ \begin{array}{l} 23.0 \\ 28.0 \end{array} \right.$	6	170.0	2.00	$\left\{ \begin{array}{l} 8 \\ 81 \end{array} \right.$
4	$\left\{ \begin{array}{l} 23.0 \\ 28.0 \\ 33.0 \end{array} \right.$	6	75.5	4.50	$\left\{ \begin{array}{l} 0 \\ 4 \\ 76 \end{array} \right.$
5	$\left\{ \begin{array}{l} 33.0 \\ 38.0 \\ 43.0 \end{array} \right.$	$\left\{ \begin{array}{l} 6 \\ 4 \\ 3 \end{array} \right.$	42.5	8.00	$\left\{ \begin{array}{l} 5 \\ 85 \\ 44 \end{array} \right.$
6	$\left\{ \begin{array}{l} 43.0 \\ 43.0 \\ 43.0 \\ 43.0 \end{array} \right.$	$\left\{ \begin{array}{l} 3 \\ 4 \\ 6 \\ 12 \end{array} \right.$	680.0	0.50	$\left\{ \begin{array}{l} 95 \\ 62 \\ 19 \\ 6 \end{array} \right.$

were centrifuged (see table 1 for accelerations used) in a chamber which was at approximately the same temperature as that of the bath. The data were obtained by determining the percentages of filaments in the cells of which the chloroplasts had been displaced (chloroplasts moved to the end of the cell). For each experimental group about 300 filaments were used. Previous experiments have

demonstrated that at 43° C. about 2 minutes is required for the temperature equilibrium to be reached in the cotton wads.

Data

Consider the change in elasticity, as evidenced by a decrease in the value of c_0 , between temperatures of 1° and 38° C. It will be noted (experiment 1) that at 1° C. the chloroplasts were displaced in only 1 per cent of the filaments. This means that in 99 per cent of them the value of c_0 was equal to or greater than $1062 \times$ gravity, and accordingly the chloroplasts would not move in response to a centrifugal acceleration of 1062 because:

$$V = k(1062 - 1062) = 0 \text{ or } V = k(1062 - > 1062) = < 0.$$

On the other hand, at 38° C. (experiment 5) c_0 was less than $42.5 \times$ gravity for the 85 per cent in which the chloroplasts were displaced and equal to or greater than $42.5 \times$ gravity for the 15 per cent of the filaments in which the chloroplasts were not displaced. According to theory, for 85 per cent of the filaments,

$$V = k(42.5 - < 42.5) = > 0.$$

Table 1 also shows that up to 38° C. as the temperature increased the effective acceleration decreased. This means that between temperatures of 1° and 38° C. the value of c_0 decreased, indicating that as the temperature increased the protoplasmic elasticity decreased. At 23° C. the value of c_0 was less than $170 \times$ gravity for only 8 per cent of the filaments (experiment 3), but at 43° C. c_0 was less than $42.5 \times$ gravity for 44 per cent of the filaments (experiment 5). This indicates that following 3 minutes' immersion at 43° C. the elasticity of the protoplasm in many cells was less than in most cells maintained at 23° C. Experiment 6 demonstrates that at 43° C. exposures greater than 3 minutes caused a coagulation of the protoplasm. Perhaps in the 56 per cent (100 - 44 per cent) of the filaments in which no movement of the chloroplasts was observed after 3 minutes' immersion (experiment 5) the protoplasm had coagulated during the 20 seconds required to transfer the tubes to the centrifuge or during the first minute of centrifugation before a noticeable displacement of

the chloroplasts had occurred. The 44 per cent in which the chloroplasts were displaced certainly indicate that a decrease in elasticity preceded coagulation. What occurred in 44 per cent of the filaments undoubtedly occurred in all, but the time required to transfer the tubes and to centrifuge the filaments militated against an actual determination of this.

To summarize, the data indicate that as the temperature was raised from 1° to 38° C., the elasticity of the protoplasm in cells of *Spirogyra* decreased. At 43° C., coagulation was preceded by a decrease in elasticity.

Discussion

Elastic fluids, such as the protoplasm in which the chloroplasts of *Spirogyra* are imbedded, are presumably made up of interlaced or variously united linear molecules.

FREY-WYSSLING (4) has deduced that protoplasm must have such a structure. He thinks that the basic or acidic end groups of long protein molecules combine with organic or inorganic ions, and that certain end groups of side chains combine with either hydrophilic or lipophilic molecules to produce a molecular complex. The molecular complexes combine with one another to produce a structural network. According to FREY-WYSSLING, the molecular complexes can unite through homopolar cohesions between neighboring alkyl groups, through heteropolar cohesions between neighboring hydroxyl groups, through heteropolar valence combinations between basic and acidic groups, or through valence combinations between reduced alkyl or sulph-hydril groups.

Figure 1 shows the possible arrangements of protein and lipid molecules. Solid lines represent proteins and dotted lines lipoids. Carbohydrates have not been represented but they may be combined with proteins or exist in solution in the interstices. Salts may be combined with the proteins, lipoids, or carbohydrates, or exist in solution in the interstices. The structure as represented in the diagram accounts for the observed elasticity. In order to move an object (such as a chloroplast) suspended in such a fluid, a definite shearing stress, c_0 , must be exceeded.

Temperature may alter the structure represented in the diagram

in various ways. It may decrease or break some cohesions. FREY-WYSSLING states that the homopolar cohesions between alkyl groups are very sensitive to temperature. Temperature may break some valence combinations or it may alter the shape of some molecules, particularly protein molecules. The separation of network constituents by a rise in temperature will loosen the structure and concomitantly decrease the elasticity of the protoplasm, which is in accordance with the data presented.

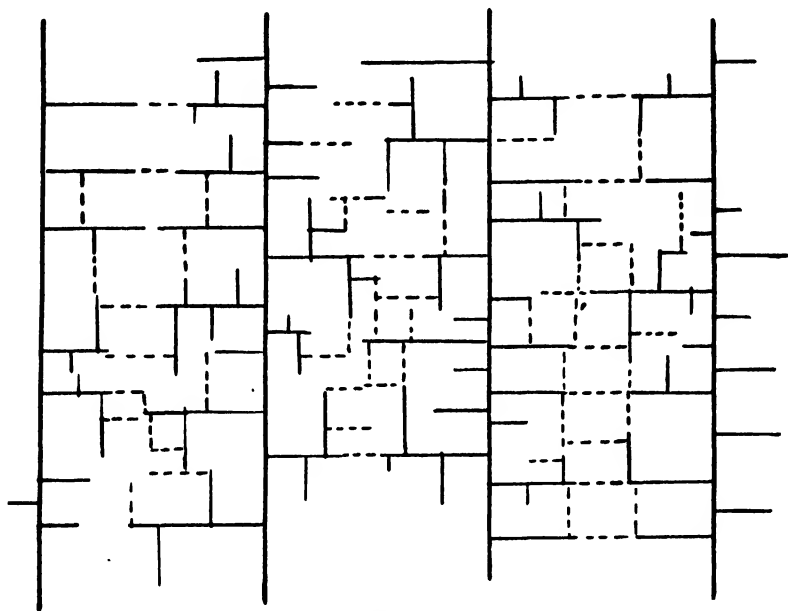


FIG. 1

The release of molecules from the network or the loosening of the network may help to explain the breaking of rest periods in plants. It is significant that warm baths, injury (10), and ether in certain concentrations and exposures (8) decrease protoplasmic elasticity, and that all of these treatments break rest periods.

The following deals with speculations on the nature of the plasma membrane. It may have a structure comparable with that of the diagram but perhaps more compact. Such a membrane may be considered a mosaic of lipoids and proteins, as BROOKS (2) has postulated. It is reasonable to think that such a membrane would be

affected by temperature as would the protoplasm in which the chloroplasts of *Spirogyra* are imbedded. If the preceding ideas are correct, an increase in temperature would loosen the network, increase the size of the pores, and increase the rate of permeability of at least those substances whose entrance is governed by pore size. It is known that, within limits, the higher the temperature the greater the rate of permeability (1). Many fat solvents enter cells rapidly. NORTIEN (8) has demonstrated that many fat solvent anesthetics in certain concentrations and exposures decrease protoplasmic elasticity in *Spirogyra*. The change in structure which the fat solvents induce may be responsible in part for the rapidity with which they enter. The present concept of the membrane also explains why such anesthetics should increase the rate of permeability of substances other than fat solvents (5). As previously mentioned, fat solvent anesthetics decrease elasticity and loosen the protoplasmic network. The concept also explains why lethal temperatures destroy the selective permeability of the membrane. Presumably such temperatures destroy the protoplasmic network.

The mechanism of heat death will be developed from the fact that a decrease in elasticity precedes coagulation. The suggested mechanism is a combination of the lipid liberation and protein coagulation theories as discussed by BĚLEHRÁDEK. The first effect of lethal temperatures is to liberate the lipoids from their combinations with proteins in the protoplasmic network. This liberation loosens the structure and accounts for the observed decrease in protoplasmic elasticity. The liberated lipid molecules fuse to form droplets, and the protein molecules, no longer separated in the network, fuse to form a coagulum. The heat itself may cause the coagulation; or the heat working synergistically with the salts present in the interstices or liberated from the network may cause the protein coagulation. That salts may be liberated has been demonstrated by MAZIA and CLARK (6). They found that a temperature of 40 °C. releases free calcium in *Elodea* cells. When the network is destroyed the solutions in the interstices form vacuoles, and vacuolization is known to accompany heat death (1).

According to the preceding hypothesis, conditions or substances

which decrease elasticity (loosen the structure) should accelerate heat death. As previously mentioned, anesthetics and mechanical injury decrease elasticity. The data of FEICHTINGER (3) suggest that mild radium treatment decreases protoplasmic elasticity. BĚLEHRÁDEK reports that anesthetics, mechanical injury, and radiations hasten heat death.

Conversely conditions which increase elasticity (tighten the network) should increase the resistance to heat. NORTHEN (9) has found that the elasticity of the protoplasm in cells of rye coleoptiles increases during drought. BĚLEHRÁDEK reports that the lower the water content of cells the greater the resistance to heat.

The hypothesis may also explain why pseudopods are withdrawn when certain organisms are exposed to high temperatures (1). Surface tension and the elasticity of the surface of such organisms tend to pull the pseudopods into the main body so that the organism will assume the shape of least free energy. Under normal conditions the pseudopods are maintained because of their structural network. When this network is destroyed by high temperature, no support remains and surface tension withdraws the pseudopods.

Summary

1. Filaments of *Spirogyra* were immersed for different periods in water maintained at various temperatures, and were centrifuged at these temperatures with different accelerations.

2. The data indicate that between 1° and 38° C. an increase in temperature lowered the effective centrifugal acceleration. For example, the low acceleration of $42.5 \times$ gravity caused displacements of the chloroplasts in cells of many filaments maintained at 43° C. for 3 minutes and at 38° C. for 6 minutes, but in practically none of those maintained at 33° C. An acceleration of $75.5 \times$ gravity caused significant displacements in cells maintained at 33° C. but not in cells maintained at 28° or at 23° C.

3. Between 1° and 38° C. an increase in temperature decreased the value of c_0 in the equation $V = k(c - c_0)$, which approximately governs the rate with which the chloroplasts in cells of *Spirogyra*

move in response to centrifugal accelerations. A decrease in c_0 was interpreted as a decrease in protoplasmic elasticity.

4. At 43° C. coagulation is preceded by a decrease in protoplasmic elasticity.

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GROWTH AND METABOLISM OF BEAN CUTTINGS SUBSEQUENT TO ROOTING WITH INDOLEACETIC ACID

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(WITH SIX FIGURES)

Introduction

Several investigations (1, 2, 4, 6, 10) have demonstrated that the application of beta-indoleacetic acid to cuttings or entire plants affects their metabolism and rate of transfer of carbohydrate and nitrogenous compounds. In a recent study (11) it was shown that the rate of transfer of these compounds, from the top downward to the hypocotyls where roots were initiated, was definitely accelerated by the application of indoleacetic acid to the bases of bean cuttings. The treated cuttings responded by rapid swelling of the hypocotyls, increased root production, and a temporary suppression of top growth. The investigation referred to was limited to a study of the responses that occurred during a period of 120 hours after treatment, the time necessary for the initiation and emergence of roots.

In connection with this earlier work, the present experiments were undertaken to compare the growth and the carbohydrate and nitrogen metabolism of treated and untreated cuttings subsequent to the time of root emergence. Several thousand cuttings were treated with indoleacetic acid solutions of two different concentrations and then grown, together with untreated cuttings, for 2 weeks in a greenhouse. The fresh weight, dry weight, and the carbohydrate and nitrogen content of various parts of the treated and untreated plants were compared at 2 day intervals during a 10 day period following root emergence.

Investigation

MATERIALS AND TREATMENT.—Approximately 8000 seedlings of kidney beans, *Phaseolus vulgaris*, were grown in soil in a greenhouse

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on two adjacent benches. On the tenth day following planting of the seeds, the plants were 5-7 inches in height, the primary leaves had expanded to approximately two-thirds of their final size, but the second internodes had not elongated appreciably and the trifoliate leaves were not expanded. The cotyledons had decreased in size, and although measurements were not made, it appeared that most of the food materials they originally contained had been translocated. During this 10 day period the temperature in the greenhouse varied between 70° and 85° F. and the light intensity from 2000 to 5000 foot candles.

Each bench was marked into two equal parts, and approximately 5200 cuttings were made October 14 by cutting selected plants through the hypocotyls a measured distance of 2 inches below the cotyledons, and collecting plants in equal numbers from each section of the benches. The cotyledons were removed; and to prevent wilting, groups of approximately 250 cuttings were immediately placed in galvanized iron treating trays containing tap water to a depth of approximately 1 inch. The cuttings were divided into three groups of equal numbers and two of these groups were treated for 3 hours by immersing the hypocotyls in solutions of indoleacetic acid dissolved in tap water. The first group was treated with a 0.002 per cent solution; this was known to be near the lowest concentration effective in stimulating root production. The second group was treated with a 0.01 per cent solution which was known to be strongly effective in stimulating root production. The remaining group was treated with tap water for an equal interval of time and used as a control.

The indoleacetic acid solutions were prepared by dissolving the required amount of acid in 10 cc. of 95 per cent alcohol. The alcoholic solutions were then added to 9 liters of water, and placed in the treating trays where the cuttings stood, during the 3 hour period of treatment, at room temperature in diffused light. They were then planted to a uniform depth of 2 inches in unsterilized quartz sand contained in 4 inch clay pots. Four cuttings were placed in each pot, and the sand was carefully washed around the hypocotyl. For the next 5 days the humidity of the surrounding air was maintained between 65 and 85 per cent by inclosing the sides and top of each bench with glass. The sand was kept moistened with tap water and the

plants were protected from intense light, the maximum intensity being approximately 1000-2000 foot candles during the first 5 days, and 5000-8000 foot candles during the remainder of the experiment, except on the ninth and tenth days after treatment when clouds re-

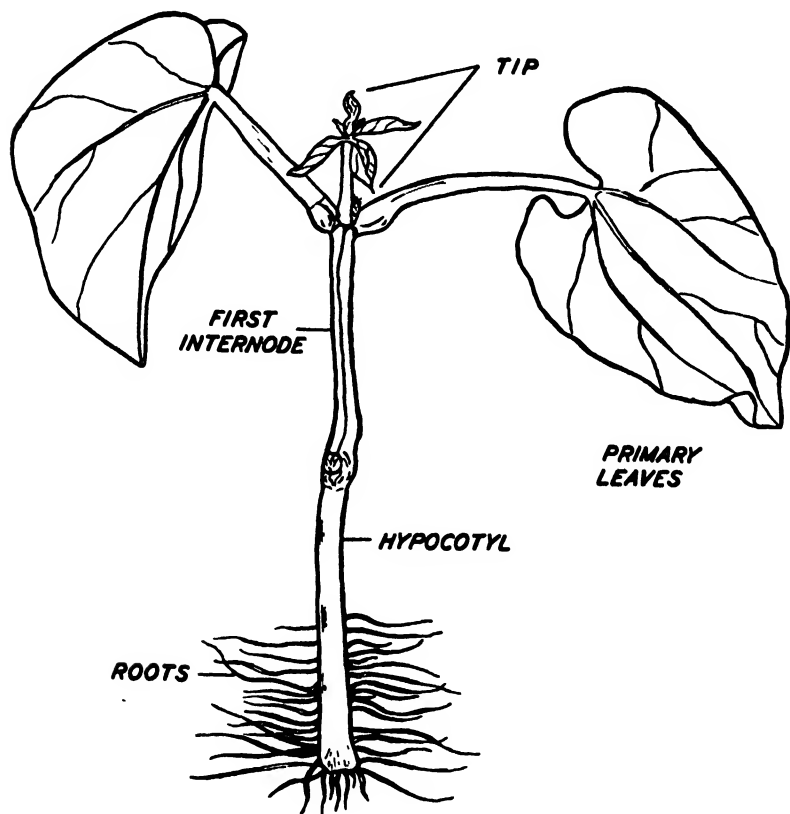


FIG. 1.—Parts into which plants were divided for weight determinations and chemical analyses. Diagram shows appearance of cutting 7 days after treatment with 0.01 per cent indoleacetic acid.

duced the intensity. The temperature generally varied between 70° and 85° F. but on one occasion reached 90° F. for a short period of time. The pots were arranged on two parallel adjacent benches that were marked into eight sections of the same size. An equal number of plants of each treatment were evenly distributed throughout each section.

On the fifth day following treatment, roots had emerged from the hypocotyls and the first harvest was made. Individual samples were systematically collected from pots in corresponding positions in each of the eight sections. The sand was carefully washed free from the roots, the plants divided into five parts (fig. 1), and the fresh weight of each part recorded. The original eight samples were then combined so that there were quadruplicate samples of each of the five parts. The tissue was dried in a well ventilated oven at 80° C. and the dry weights recorded. The samples were again combined, so that duplicate samples of each part were used for chemical analyses.

TABLE 1
ANALYSIS OF VARIANCE OF DRY WEIGHT DATA FROM TABLE 3

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE FOR PARTS			
		HYPOCOTYLS	FIRST INTERNODES	TIPS	ROOTS†
Total	71	0.0634	0.0735	19.847	0.2984
Treatment	2	0.6514*	0.1688*	3.370*	0.8180*
Plot	3	0.0213	0.0065	1.688*	0.0748
Time	5	0.3722*	0.8842*	277.140*	3.6120*
Remainder (error)	61	0.0209	0.0072	0.1918	0.0259

* Significantly greater than error of variance by odds of more than 99 to 1.

† Degrees of freedom: total 59; time 4; and remainder 50.

One of these samples was therefore made up of plants from one bench and one of plants from the other.

Subsequent harvests were made on every second day for a period of 10 days. During this interval the unharvested plants were supplied, on days between sampling, with a complete nutrient solution (8). The dry weight data for hypocotyls, first internodes, tips and roots were analyzed statistically for variance (table 1), and the difference in grams of dry matter per mean sample necessary for significance was determined (table 3).

CHEMICAL METHODS.—Two separate samples, obtained as previously described, were ground to pass a 40-mesh sieve and analyzed. The moisture content was determined by drying duplicate portions at 100° C. Sugars were extracted with 80 per cent alcohol in Soxhlet. The reducing power of the dealeded extracts was determined

before and after inversion with invertase by the Munson and Walker method, followed by titration with potassium permanganate. Direct reducing sugars were expressed as glucose and total sugars as invert sugar. Sucrose was calculated by multiplying the difference between total sugars and reducing sugars (as invert) by 0.95.

Total nitrogen was determined by the reduced iron modification of the Gunning method (9), to include nitrates. Analysis was made for the insoluble nitrogen in the residue remaining after extraction of the sugars by the Gunning method, and a correction made for the extracted weight of the samples.

TABLE 2

EFFECT OF INDOLEACETIC ACID TREATMENT ON NUMBER AND DRY WEIGHT OF ROOTS PRODUCED BY BEAN CUTTINGS. DRY WEIGHTS AND ROOT COUNTS REPRESENT 200 PLANTS. THE NUMBER OF ROOTS ON 50 PLANTS OF EACH TREATMENT WERE COUNTED

TREATMENT	DRY WEIGHT 7TH DAY (GM.)	DRY WEIGHT 15TH DAY (GM.)	INCREASE IN DRY WEIGHT (GM.)	AVERAGE NUMBER OF ROOTS 15TH DAY	AVERAGE DRY WEIGHT PER ROOT 15TH DAY (MG.)
Control.....	1.2	6.7	5.5	9800	0.68
0.002%.....	1.3	6.3	5.0	9700	0.65
0.01%.....	2.7	7.1	4.4	15960	0.44

Results

GROWTH RESPONSES.—The hypocotyls of cuttings treated with a 0.01 per cent solution of indoleacetic acid increased in size more rapidly during the first week following treatment than did those treated with 0.002 per cent or the controls. Most of the roots emerged on the fifth day. Relatively fewer roots were produced by the control and weaker treatment, and these were clustered toward the lower end of the hypocotyls, while plants treated with the stronger solution produced roots in great numbers (table 2), which in general were developed over a considerable portion of the hypocotyls. Although plants treated with the 0.01 per cent solution produced more roots, their average size appeared to be less than that of either those of controls or those of the plants that were treated with the less concentrated solution.

There was an evident increase in the height and leaf area of the controls and also the plants treated with 0.002 per cent acid solution during the 5 days immediately following treatment. Those treated with the more concentrated solution, on the other hand, showed no appreciable increase in height or leaf area until the seventh day. On the eleventh day following treatment, control plants were approximately 8-10 inches in height, those treated with 0.002 per cent solution 6-8 inches, and those treated with 0.01 per cent solution 4-6 inches above the ground level. This difference became less apparent toward the end of the experiment, and on the fifteenth day there was little difference in the height or general appearance of the plants in all three treatments.

The dry weight of the roots of plants treated with 0.002 per cent acid increased by approximately the same amount as those of controls during the experiment, whereas those of plants treated with a 0.01 per cent solution contained 130 per cent more dry matter than did those of untreated plants, 7 days after treatment (table 2). The roots of untreated plants grew more vigorously toward the latter part of the experiment, however, and at the time of the final harvest the difference in dry weight of the total number of roots on treated and untreated plants was only approximately 6 per cent (fig. 2, table 2). The average dry weight of individual roots of control plants, and also of those treated with 0.002 per cent acid, was approximately 52 per cent greater than those of plants treated with the more concentrated solution.

The dry weight of the hypocotyls of plants treated with a 0.01 per cent solution was significantly greater than that of untreated plants during the early part of the experiment, but this difference also decreased until the amount of solid matter in hypocotyls of treated and untreated plants was not significantly different at the time of the final harvest (table 3, fig. 2). The amount of solid matter in the first internodes of treated plants increased more rapidly than that of untreated plants only during the first 5 days following treatment (fig. 3). The dry weight of the primary leaves decreased approximately the same amount in all treatments during the experiment, and this decrease was most rapid during the 5 days immediately following treatment (fig. 3). The dry weight of the tips

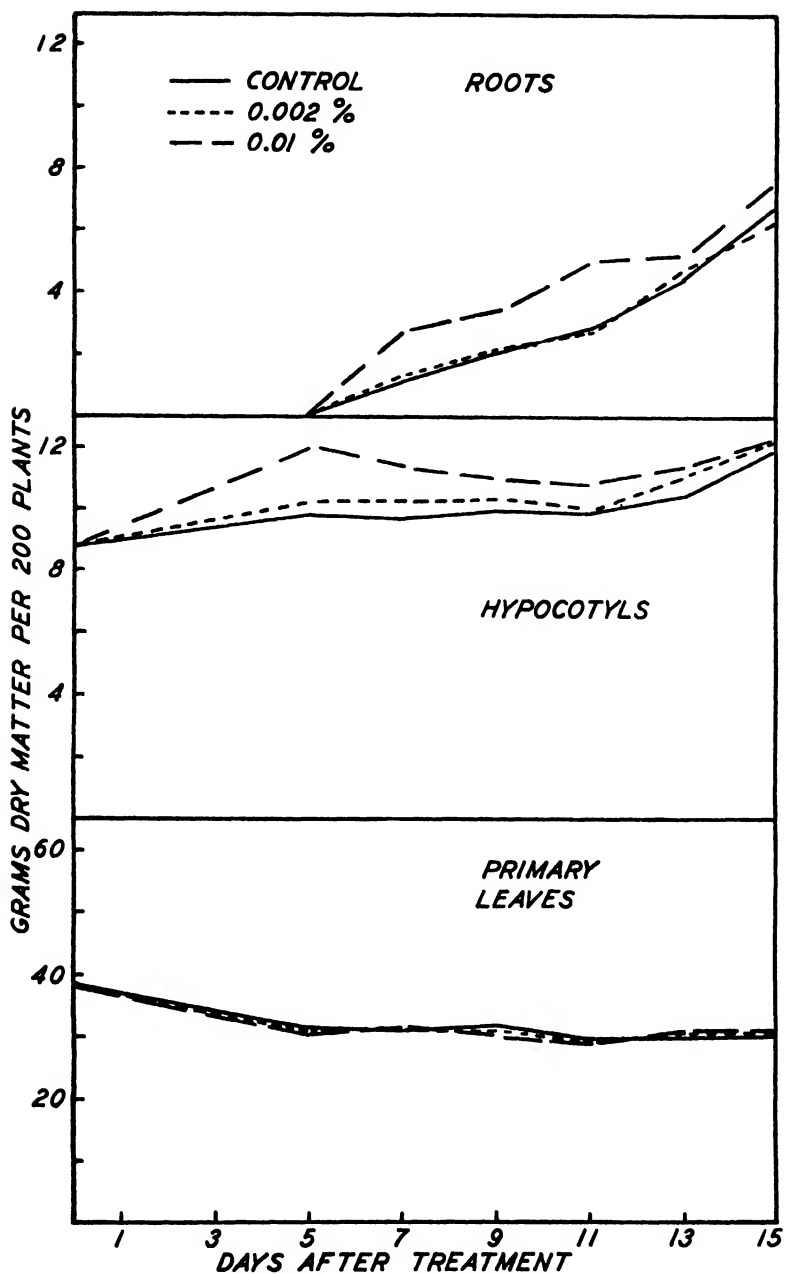


FIG. 2.—Dry weights of various parts of cuttings treated with indoleacetic acid solutions as compared with those of untreated plants.

of control plants increased more rapidly and became significantly greater than that of treated plants by the ninth day following treatment (fig. 3), and this difference remained significant throughout the remainder of the experiment.

TABLE 3

DRY WEIGHTS* OF VARIOUS PARTS OF BEAN PLANTS GROWN IN QUADRUPLICATE PLOTS AND TREATED WITH 0.002 PER CENT (WEAK) AND 0.01 PER CENT (STRONG) SOLUTIONS OF INDOLEACETIC ACID, AS COMPARED WITH WEIGHTS OF SIMILAR PARTS OF UNTREATED PLANTS. FIGURES REPRESENT AVERAGE WEIGHT PER SAMPLE (56 PLANTS) PER PLOT

DAYS AFTER TREATMENT	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG
	ROOTS			HYPOCOTYLS			FIRST INTERNODES		
Initial check.....	2.47	1.40
5th.....	2.75	2.86	3.37†	1.96	1.88	1.80†
7th.....	0.333	0.351	.762†	2.71	2.86	3.19†	1.94	1.94	1.78†
9th.....	0.595	0.599	.949†	2.78	2.90	3.08†	2.21	2.12	2.01†
11th.....	0.81	0.805	1.39†	2.76	2.78	3.04†	2.12	2.13	1.99†
13th.....	1.24	1.31	1.49†	2.91	3.09	3.09	2.38	2.32	2.26†
15th.....	1.86	1.77	1.99	3.33	3.32	3.40	2.66	2.63	2.47†
	LEAVES			TIPS			TOTAL		
Initial check.....	10.71	0.403	14.983
5th.....	8.91	8.55	8.47	0.90	0.76	0.55	14.52	14.05	14.19
7th.....	8.68	8.76	8.75	1.64	1.54	1.13	15.303	15.451	15.612
9th.....	9.00	8.72	8.63	3.54	3.20	2.65†	18.125	17.539	17.319
11th.....	8.27	8.20	8.37	6.10	5.87	5.20†	20.06	19.785	19.99
13th.....	8.30	8.42	8.62	9.35	9.22	8.60†	24.18	24.36	24.06
15th.....	8.42	8.45	8.50	13.55	13.22	12.57†	29.82	29.39	28.93

* Differences between weights required for significance: hypocotyls 0.20; first internodes 0.12; tips 0.62; and roots 0.23 gm.

† Significantly different from controls.

The total increase in solid matter per plant during the entire experiment was not appreciably affected by treatment with the acid. During the first 5 days following treatment there was, in fact, a slight decrease in total solid matter. Table 3 shows that plants which were treated with 0.002 per cent acid lost 6.5 per cent of their original weight; those treated with 0.01 per cent solution, 5.5 per cent

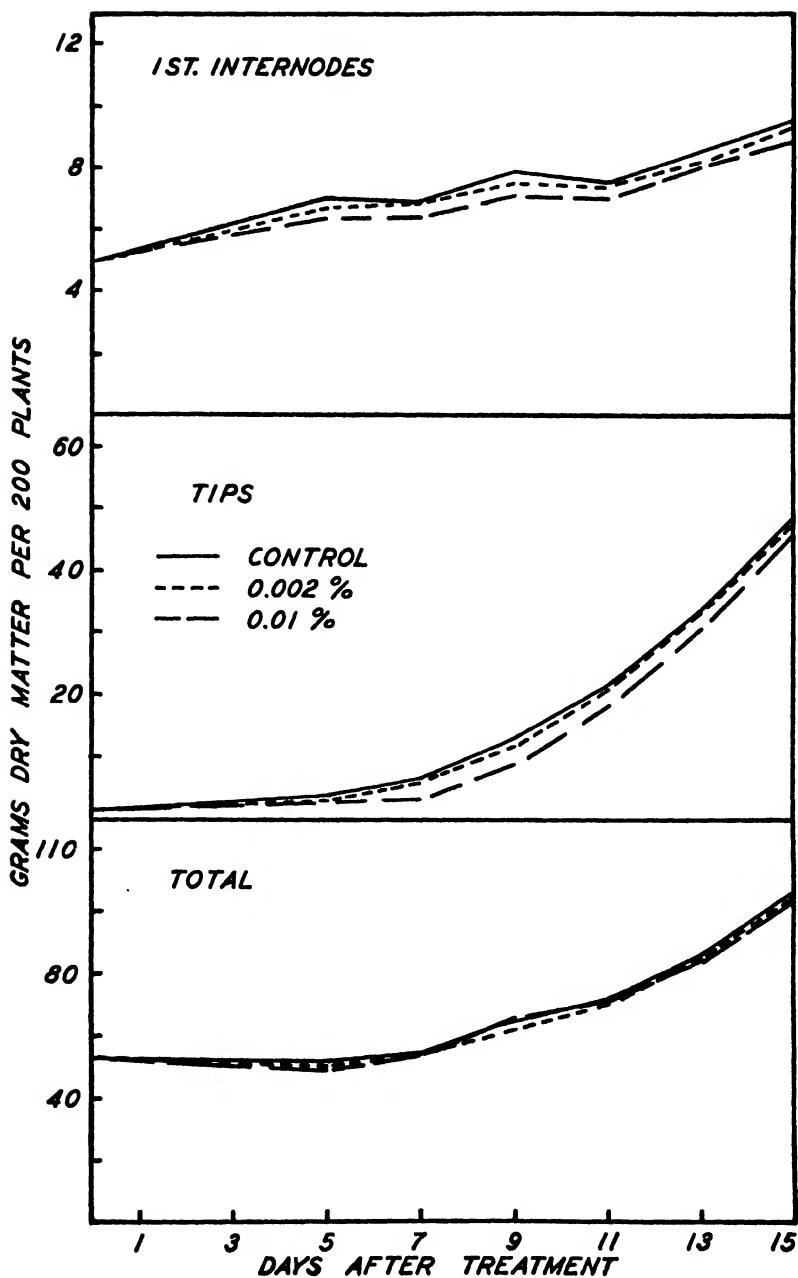


FIG. 3.--Dry weights of various parts of cuttings treated with indoleacetic acid solutions as compared with those of untreated plants.

of their initial weight; while untreated plants decreased only 3.2 per cent in dry weight during the same period of time.

There was at first a very apparent difference in the size of treated as contrasted with untreated cuttings, but this difference in appearance gradually became less and was hardly noticeable at the end of the experiment. As contrasted with this, approximately the same total amount of solid matter was synthesized by both treated and untreated plants. Thus the height and possibly the leaf area of control plants increased during the early part of the experiment more rapidly than did treated plants, but the total amount of solid matter synthesized was unaffected by treatment.

The percentage of moisture in treated cuttings was approximately the same as that of control plants between the fifth and fifteenth day after treatment, and the fresh weights of the total or of the different parts of treated and untreated cuttings show essentially the same results as have been described in connection with dry weight measurements.

In general the growth and appearance of cuttings treated with 0.002 per cent acid was not appreciably different from that of control plants at the end of a 15 day growing period following treatment. As contrasted with this behavior, the growth of cuttings treated with 0.01 per cent acid was accelerated in the region of root initiation, during the 5-7 days following treatment; the growth of the stems, petioles, and leaves was retarded for a longer period of time following planting than was that of untreated plants.

During the 5 days immediately following treatment, the total dry weight of stem tips, leaves, petioles, and first internodes of control plants decreased 6.3 per cent while that of the hypocotyls increased 11.4 per cent of their original weight. As compared with these figures, similar parts of cuttings treated with 0.01 per cent acid decreased 13.8 per cent and gained 36.4 per cent, respectively. The stimulating effect of indoleacetic acid on cell division and root initiation in the hypocotyl and the downward transport of food materials was only temporary, and these responses did not appreciably affect the synthesis of solid matter by the entire plant.

INFLUENCE OF INDOLEACETIC ACID ON METABOLISM OF CARBOHYDRATES.—It can be seen from figure 4 that the process of root for-

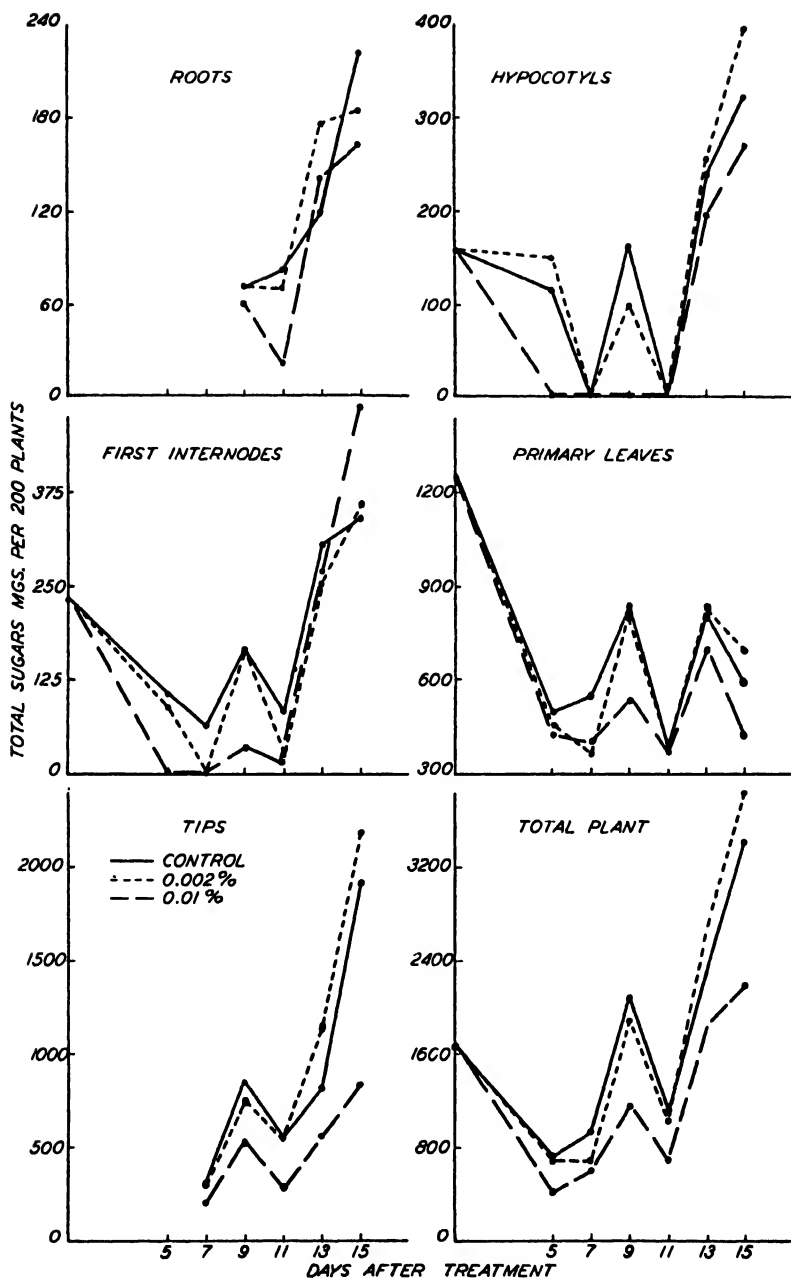


FIG. 4.—Effect of indoleacetic acid on sugar content of various portions of bean cuttings as compared with that of untreated cuttings.

mation on untreated cuttings was accompanied by a reduction in the sugar content of the cutting as a whole. This reduction was more pronounced in cuttings treated with indoleacetic acid, presumably owing to increased meristematic activity and root formation.

After root emergence on the fifth day following treatment, the leaf area increased rapidly and sugars accumulated in all of the cuttings for the duration of the experiment. The process of sugar accumulation was continuous with but one exception. Between the ninth and eleventh days a sharp reduction in sugar content took place in all the cuttings. The uniformity of this response suggested a variation in some environmental factor. While no significant difference in greenhouse temperature occurred at this time, there was a great reduction in light intensity on the ninth and tenth days after treatment. This reduction, as measured by a recording pyroheliometer, amounted to from 70 to 80 per cent of the light intensity on other days of the experiment, and is doubtless responsible for the lower sugar content of the cuttings harvested 11 days after treatment.

Table 3 shows that at the end of the 15-day growth period the treated cuttings weighed only slightly less than the controls. There were, however, large differences in the sugar content. On the thirteenth and fifteenth days the sugar content of cuttings treated with a 0.002 per cent acid was greater than that of untreated plants (figs. 4, 5). Prior to this time control plants contained slightly greater amounts of sugar. It is possible that the treatment with weak indoleacetic acid actually increased the rate of photosynthesis during the latter portion of the experiment. MITCHELL and HAMNER (7) noted that under certain conditions very low concentrations of indoleacetic acid-lanolin paste applied to the second internodes of intact bean plants stimulated synthesis of dry substance in excess of that produced by untreated plants.

Cuttings treated with 0.01 per cent acid contained less sugar than the controls throughout the course of the experiment. This lower content was not due to sugars being condensed to starch, as tests were made on all samples and starch could not be detected in the leaves, stem tips, or roots, and only traces were found in the

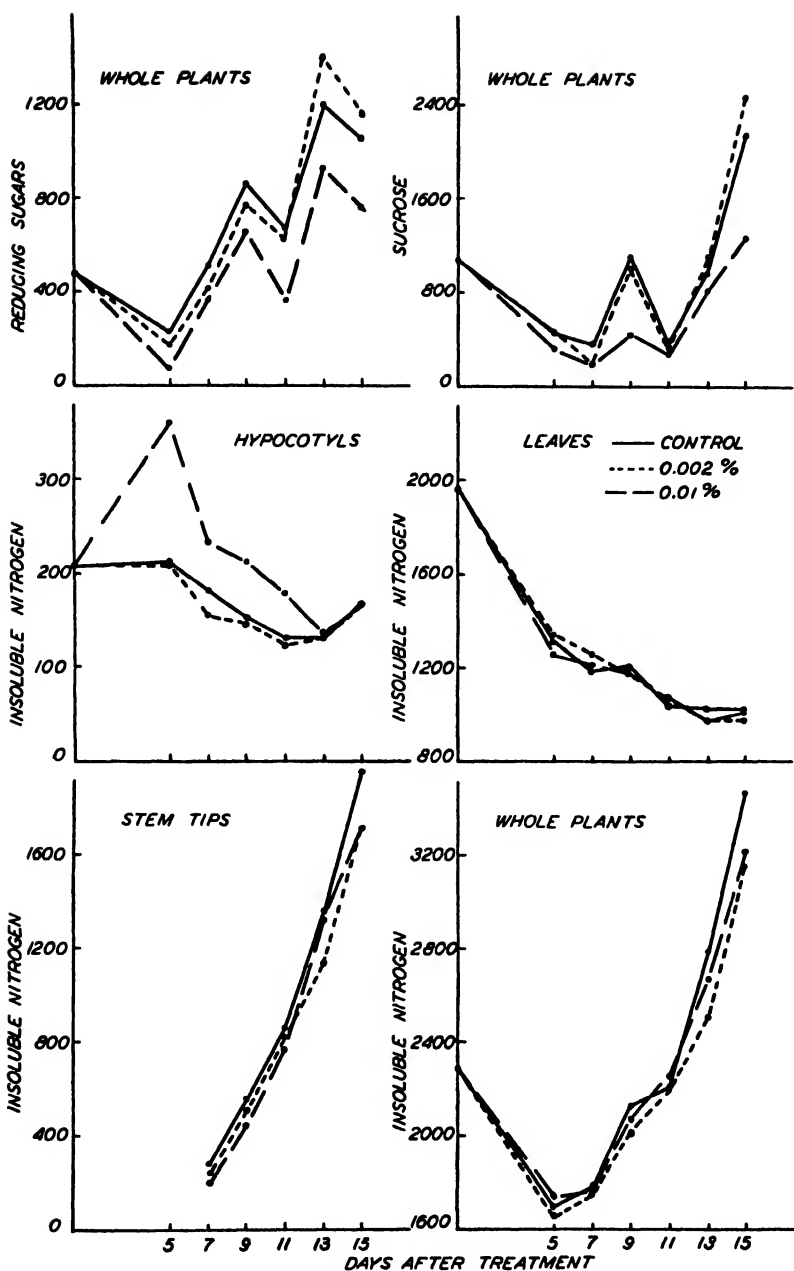


FIG. 5.—Effect of indoleacetic acid on sugar and insoluble nitrogen content of various portions of bean cuttings as compared with that of untreated cuttings. Plotted on basis of milligrams per 200 plants.

hypocotyls and first internodes. It is possible, however, that the sugars may have been condensed into polysaccharides other than starch, particularly in tissues near the point of treatment. Thus at the time of final harvest the dry weight of the roots of 200 control plants was 6.66 gm. as compared with 7.12 gm. for plants treated with 0.01 per cent acid. The amount of total sugars, on the other hand, was 222 mg. for controls and only 162 mg. for 200 treated plants. At the final harvest the dry weights and sugar content of the hypocotyls showed a similar inverse relationship. This difference between dry weight and amount of sugar could not be accounted for by nitrogen, as table 4 shows the nitrogen content of roots and hypocotyls of treated and untreated plants to be essentially the same at the time of final harvest. It is also of interest in this connection that the dry weight of the hypocotyls of plants treated with 0.01 per cent acid increased 36 per cent during the 5 days following treatment, while their sugar content decreased to approximately zero during the same interval. Although no direct measurements have been made, it is probable that the activity of enzymes concerned in the formation of certain polysaccharides, such as those present in cell walls, was accelerated through the use of indoleacetic acid. ALEXANDER (1) has reported that bean plants treated with indoleacetic acid-lanolin contained larger amounts of acid hydrolyzable polysaccharides, particularly near the region of application.

The initial cuttings contained more than twice as much sucrose as reducing sugars (table 4, fig. 5). Frequently throughout the course of the experiment sucrose was the only sugar present in certain portions of the cuttings. Thus there was no detectable amount of reducing sugars present in hypocotyls of either treated or untreated plants until the thirteenth day after treatment. Likewise, although the first internodes of the initial cuttings contained reducing sugars, none were found subsequently until the thirteenth day. Reducing sugars were always present in the leaves and predominated in the stem tips until the fifteenth day, when large quantities of sucrose were stored in the tips. The stem tips of both treated and untreated plants contained a much greater amount of sucrose and reducing sugars than did the roots of these plants.

In general the results show that when the plants were considered

as a whole the sugars decreased in all of the cuttings during the 5 day period immediately following treatment, the period when roots emerged. After the roots had emerged there was an apparent increase in the rate of leaf expansion, and sugars increased in all of the cuttings but were at all times lower in those receiving the strong treatment than in the controls (fig. 4). There is also some evidence that the weak treatment increased the sugar content, expressed either as percentage or absolute amount, over that in the control plants (figs. 4 and 5, table 4).

INFLUENCE OF INDOLEACETIC ACID ON METABOLISM OF NITROGEN.

—The cuttings were not supplied nutrient solution containing nitrogen until after they had rooted; that is, on the sixth day. During this period of root initiation and emergence, nitrogen was mobilized from the leaves to the hypocotyls of both treated and control plants, but the rate of transport was stimulated as a result of the indoleacetic acid treatment, the stronger treatment being the more effective (fig. 6). Thus the hypocotyls of plants treated with 0.002 per cent acid gained twice as much and those treated with 0.01 per cent acid gained six times as much total nitrogen as did those of untreated plants during a period of 5 days following treatment. A negligible amount of this nitrogen that accumulated in the hypocotyls of control plants, and also those treated with 0.002 per cent acid, was present as insoluble nitrogen. In contrast to this, 66 per cent of the nitrogen accumulated, during the 5 days following treatment, in the hypocotyls of plants treated with 0.01 per cent acid was converted into an insoluble form.

After the initiation and emergence of roots, nitrogen steadily decreased in all of the hypocotyls, reaching a point where they contained less than at the beginning. The decrease was most rapid in the hypocotyls given the strong treatment in which all of the mobilized nitrogen disappeared. The implication is that the treatment with indoleacetic acid increased the proteolytic enzyme activity.

The reduction in nitrogen content of the hypocotyls of cuttings that received the strong treatment was equivalent to the amount deposited in the roots produced by these hypocotyls. In the case of those given the weak treatment, however, additional nitrogen besides that withdrawn from the hypocotyls was found in the roots.

TABLE 4

COMPOSITION OF KIDNEY BEAN CUTTINGS TREATED OCTOBER 14 WITH WATER, 0.002 PER CENT (WEAK), AND 0.01 PER CENT (STRONG) INDOLEACETIC ACID FOR THREE HOURS

DAYS AFTER TREAT- MENT	ROOTS			HYPOCOTYLS			FIRST INTERNODES			LEAVES			TIPS			TOTAL
	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG	CON- TROL	WEAK	STRONG	
TOTAL NITROGEN (MG.) PER 200 CUTTINGS																
0.....				597			309			2805			104			3815
5.....				634	671	828	456	453	469	2321	2210	2156	239	207	155	3650
7.....		73	78	166	591	624	748	431	431	2124	2237	2134	406	387	297	3644
9.....		130	128	203	535	574	612	446	457	2034	1951	1084	782	727	630	3927
11.....		104	160	272	408	478	572	384	381	1753	1766	1865	1308	1295	1217	3927
13.....		239	248	284	437	461	510	349	346	1640	1718	1766	1855	1837	1789	4077
15.....		322	306	336	424	438	488	317	331	1544	1554	1601	2530	2516	2491	4520
																5137
																5145
																5240
INSOLUBLE NITROGEN (MG.) PER 200 CUTTINGS																
0.....				208			102			1970						2280
5.....				213	209	362	173	116	120	1310	1332	1259				1696
7.....				181	157	233	140	87	115	1189	1256	1215	274	251	202	1784
9.....		80	83	119	153	147	213	124	110	1209	1170	1177	504	516	452	2130
11.....		100	102	168	131	124	178	86	83	1036	1061	1064	857	826	770	2210
13.....		158	149	147	132	131	134	98	104	1030	984	975	1368	1139	1319	2786
15.....		204	207	209	172	169	170	110	93	1027	973	1010	1934	1709	1720	3467
																3151
																3215

DIRECT REDUCING SUGARS (MG.) PER 100 CUTTINGS

0.....	None	60.0	None	None	425	177	72.5	485	229	177	72.5
5.....	None	None	None	None	229	181	206	233	168	512	414
7.....	None	None	None	None	206	181	206	246	370	863	774
9.....	19.2	28.9	29.2	None	295	395	259	549	370	863	774
11.....	56.4	48.7	Trace	None	230	190	167	387	390	196	673
13.....	72.8	95.9	100	None	411	484	394	487	655	331	1202
15.....	148	122	120	127	92.3	69	9	451	346	251	1061
				150	174	93		367	176	354	1167
				128	178			264			768

SUCROSE (MG.) PER 100 CUTTINGS

0.....	151	161	Trace	85.5	Trace	774	235	250	326	1086	446
5.....	110	145	Trace	None	None	241	153	169	63.1	59	8
7.....	None	None	None	None	None	497	457	246	278	138	1135
9.....	46.8	38.7	27.5	155	34.2	142	107	164	141	126	379
11.....	20.4	17.0	20.5	Trace	None	75.2	22.1	17.7	274	300	435
13.....	37.3	70.2	31.8	123	154	161	147	165	307	274	196
15.....	63.9	52.0	34.2	184	226	131	150	170	373	294	289
											212
											1469
											1713
											538
											2161
											4450
											1288

TOTAL SUGARS (MG.) PER 100 CUTTINGS

0.....	159	235	Trace	89.5	Trace	1272	496	457	426	1666	718
5.....	116	152	Trace	None	None	545	302	403	324	397	198
7.....	None	None	None	None	None	834	806	539	861	746	530
9.....	70.9	72.3	60.7	103	102	162	36.3	33.9	555	541	291
11.....	81.8	70.0	21.5	Trace	None	79.7	23.6	18.4	380	339	1110
13.....	118	176	140	240	257	304	253	249	808	704	1130
15.....	222	184	102	325	395	344	363	492	589	606	418
											1920
											2181
											842
											3400
											3819
											2187
											1666
											718
											609
											669
											601
											933
											2006
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											1015
											1015
											600
											2297
											2655
											1848

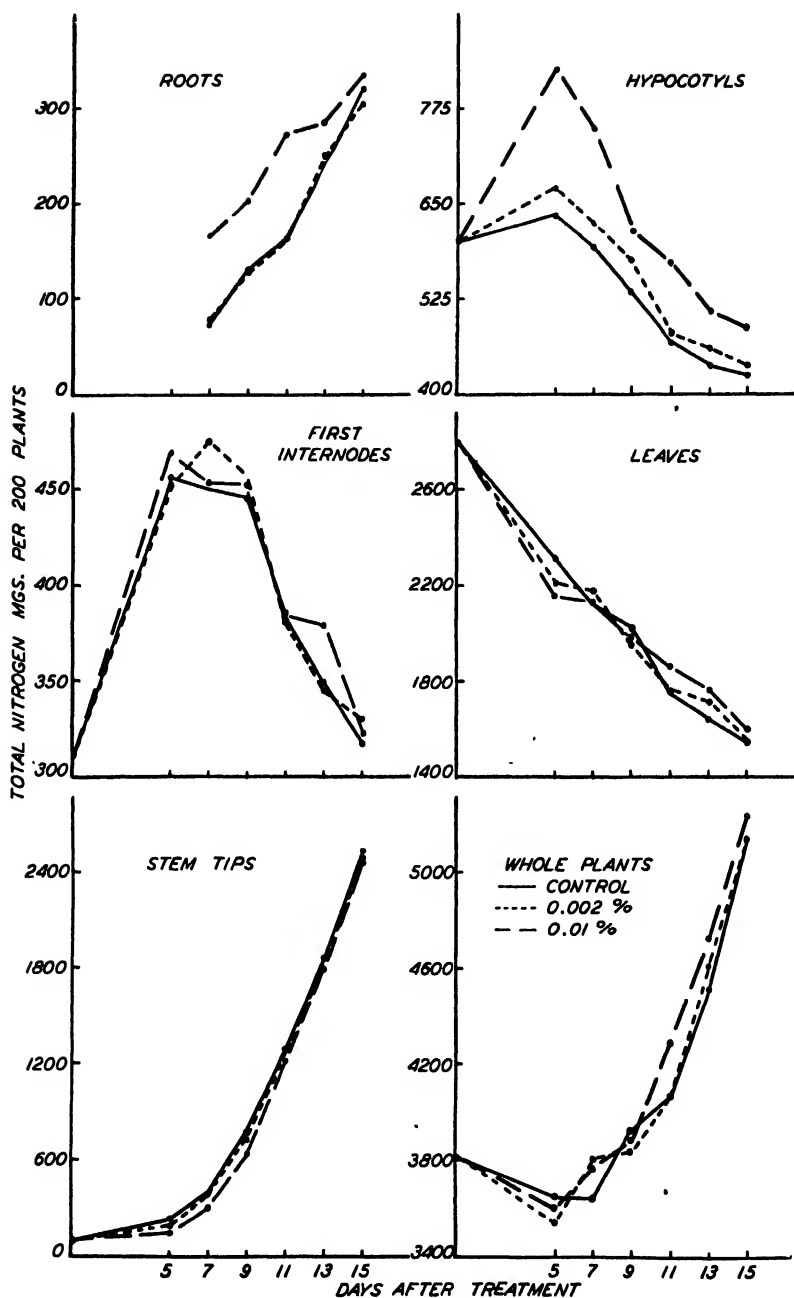


FIG. 6. —Effect of indoleacetic acid on total nitrogen content of various portions of bean cuttings as compared with that of untreated cuttings.

Since nitrogen was supplied in the nutrient solution after the roots had emerged, the exact source of the nitrogen in the roots is uncertain.

Nitrogen from the leaves accumulated also in the first internodes of all the cuttings during the initiation and emergence of roots. In contrast to the nitrogen content of the hypocotyls, the accumulation was nearly as great in the controls as in the treated cuttings. After the roots had emerged, nitrogen was translocated from the first internodes to other parts of the cuttings (fig. 5).

It is of interest that during the period of root initiation some of the nitrogen which was translocated from the leaves moved to the hypocotyls and first internodes. After root emergence, however, the nitrogen which continued to move from the leaves apparently entered the growing stem tips (fig. 5). The withdrawal of nitrogen from the leaves was continuous throughout the experiment, even after additional nitrogen from the nutrient solution was supplied through the roots. However, figure 5 shows that there was very little decrease of insoluble nitrogen (protein hydrolysis) in the leaves after the eleventh day. By this time the roots had developed sufficiently to absorb appreciable amounts of nitrogen from the nutrient solution.

The nitrogen content of the tips increased from 3 per cent of the total nitrogen in the initial cuttings to almost 50 per cent of the nitrogen in the plants at the end of the experiment. Treatment with indoleacetic acid resulted in slightly less growth of the tips than in the controls. As a result, the total amount of nitrogen in the tips from the treated cuttings was always less than in the control tips. This difference decreased after the ninth day and at the end of the experiment was less than 2 per cent.

Applications of nitrogen as a nutrient increased the nitrogen content of the cuttings by about 35 per cent. The amount of nitrogen absorbed by cuttings treated with 0.01 per cent acid was not appreciably greater than that absorbed by control cuttings, although approximately 60 per cent more roots emerged from hypocotyls of the former. Ash determination made on the tips and leaves of the plants at the end of the experiment showed that 200 treated plants contained but 520 mg. more ash than similar portions of control

plants. The increased root production did not appreciably influence the uptake of nutrients. It should be pointed out that although plants treated with the strong acid produced a greater number of roots than did the controls, the dry weight of roots of cuttings that received the stronger treatment was only slightly greater than that of the controls at the end of the experiment.

The slight difference in weight in favor of the control plants at the end of the experiment is reflected chiefly in the tips. This result is attributable, in part at least, to an influence of the acid on the carbohydrate metabolism of the cuttings. In so far as the nitrogen metabolism is concerned, the indoleacetic acid effect is most noticeable during the process of root initiation and emergence.

Discussion

It is known that some internal factors can modify the response of plants to indoleacetic acid. Thus the duration of increased protoplasmic streaming induced in the cells of some plants by low concentrations of auxin is affected by the amount of available sugar (12). The growth of oat coleoptiles, induced by the application of auxin, is also dependent upon available sugars and possibly upon the presence of certain salts (13). It is probably true that many other organic and inorganic substances generally classed as nutrients might also modify the response of certain plants to auxin treatment, although there is at present little proof of this assumption. Experiments on the rooting of cuttings (3, 14) have shown that indoleacetic acid possibly acts as a mobilizer of naturally occurring root forming substances, and that the lack of these substances could also limit the rooting response of a cutting to indoleacetic acid treatment.

Recent investigations have shown that if all of the necessary factors are present, and indoleacetic acid is applied in appropriate concentrations to some plants in the form of a paste, then many of the cells near the point of treatment increase in size, their protoplasm becomes more dense, and cell division occurs (5). If this chain of reactions which leads to the formation of new cells is initiated in one portion of the plant, by the use of indoleacetic acid, then the necessity for an adequate supply of foods in that part becomes, temporarily at least, very great. When the acid is applied to cuttings which

root readily without treatment, such as those of kidney beans, then the processes of cell division and root formation are stimulated above that of untreated cuttings, and there is necessarily a need for additional nutritive substances out of which new cells can be formed in the treated portion. Thus in the experiments reported here, carbohydrate and nitrogenous compounds moved from even remote parts of the plants to the point where cellular division was stimulated. This movement of compounds from one part of the plant to be combined into new cells formed in another part becomes an important factor, particularly in connection with the rooting of cuttings, as it concerns the general nutrition and growth of the plant.

It is therefore of considerable practical importance to determine whether the propagation of cuttings with growth substances affects their subsequent growth and behavior after rooting. An earlier paper (11) discussed the effect of treatment with indoleacetic acid on the nitrogen and carbohydrate metabolism of kidney bean cuttings during the rooting process. The present experiment was planned to secure information concerning the effect of the acid on the growth and metabolism of bean cuttings after they were rooted.

In order to evaluate the effect of the treatment on growth of the cuttings, it was necessary to set up the experiment in such a manner as to permit statistical interpretation of the growth data. These data show that neither of the concentrations here used increased the growth of the cuttings in comparison with the controls. It is possible, of course, that cuttings of other species might react differently. As previously pointed out, there was an initial stimulation in growth of the roots and hypocotyls as a result of treatment, accompanied by less growth of the stem tips and first internodes on the treated cuttings during the rooting process. It was during this period that striking differences in distribution of nitrogen and carbohydrates were noted. The difference between the weak and strong treatment, as well as results previously obtained (11), indicates that this initial growth response is dependent upon the strength of the indoleacetic acid as well as the length of exposure of the cuttings to it.

The initial influence of the acid was modified during the later growth of the plants. Thus at the end of the experiment there was an insignificant difference between the weights of the various parts

of the treated cuttings and the corresponding parts of the controls, with the exception of the stem tips and first internodes. This difference in growth was not due to a deficiency of nitrogen, since the treated cuttings contained slightly more nitrogen than the controls. As far as the carbohydrates are concerned, the weak treatment with indoleacetic acid resulted in an apparent increase of sugar content, while the strong treatment significantly reduced the sugars in the cuttings in comparison with the controls. It is not possible to interpret these findings with respect to the carbohydrate metabolism fully until the relative influences of the acid on the rate of photosynthesis, respiration, and enzymatic activity are better understood.

The present investigation shows that treatment of kidney bean cuttings with indoleacetic acid results in a prompt and distinctive growth response until the cuttings are rooted. After that period, the stimulating and modifying effects of the acid on growth tend to diminish during the subsequent vegetative development of the plants until little difference exists between the treated and control plants at the end of the experiment.

Summary

1. Cuttings of kidney bean seedlings were treated by immersing their bases in water, 0.002 per cent, or 0.01 per cent indoleacetic acid for 3 hours. They were then set in quartz sand contained in 4 inch clay pots. The pots were systematically distributed on each of two greenhouse benches which were then inclosed by glass to maintain a relatively high humidity. Cuttings were harvested 5 days after treatment and every second day thereafter until 15 days had elapsed. Each sample was divided into roots, hypocotyls, first internodes, primary leaves and petioles, and tips. Fresh weight, dry weight, total and insoluble nitrogen, reducing sugars, and sucrose were determined for each sample.

2. Treatment with 0.01 per cent indoleacetic acid significantly increased the weight of the hypocotyls and roots over the corresponding portions of the controls until the thirteenth day after treatment. The same treatment significantly decreased the growth of the first internode throughout the course of the experiment and also the

growth of the tips, and the difference became statistically significant on the ninth day and remained approximately constant through the rest of the experiment. Treatment with even a strong solution (0.01 per cent) of acid did not appreciably affect the dry weight of the primary leaves. Weak treatment (0.002 per cent) caused slight growth responses that were qualitatively like the strong treatment but statistically insignificantly different from the controls. Neither the weak nor the strong treatment increased the weight of the whole plant above that of the control.

3. During the rooting process nitrogen was transported from the leaves to the first internodes and hypocotyls, the strong treatment being by far the most effective in increasing the amount mobilized. This nitrogen was subsequently translocated to other portions of the cuttings. In the case of cuttings treated with 0.01 per cent acid, a large amount of this mobilized nitrogen was temporarily deposited in the hypocotyls in an insoluble form, but it was subsequently translocated to other portions of the cuttings. Since all of this accumulated nitrogen subsequently disappeared, it is evident that the treatment stimulated the proteolytic enzymatic activity of the cuttings.

4. Application of nutrient solution after the cuttings were rooted increased their nitrogen content by about 35 per cent. The larger number of roots induced as a result of treatment resulted in the uptake of only a slightly greater amount of nitrogen and inorganic substances than were absorbed by the controls.

5. The treatment of cuttings with 0.002 per cent acid resulted in slightly greater accumulation of sugars than in the controls at the end of the experiment. Treatment with 0.01 per cent acid significantly reduced the sugar content of cuttings below that of controls at all times. No starch was present at any time in the roots, leaves, or tips, and only traces in the hypocotyls and first internodes. Interpretation of the carbohydrate metabolism in relation to growth requires further information as to the effect of the acid on the rates of photosynthesis, respiration, and enzymatic activity.

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greatly different from one another in pod yield. The individual pods, however, were somewhat heavier on the 12 hour photoperiod.

DISCUSSION

In previous work (3) it has been shown that flower primordia can be initiated by Biloxi soy beans under a wide range of photoperiods. The critical photoperiod above which they are not initiated is about 14 hours. In the present work the photoperiods that have previously been shown to be most favorable for initiation are likewise most favorable for subsequent growth and development of flower primordia that have already been initiated. The flower buds grew much faster on the plants receiving photoperiods of 8 to 13 hours than on those receiving longer photoperiods. Continuous but slow growth of the buds occurred on 14 hour photoperiod and all of the plants finally bloomed. On 15 hour photoperiods a similar effect was observed, but at the time the experiment was discontinued not all of the plants had bloomed.

Initiation of flower buds has previously been observed on 14 hour photoperiod in the Biloxi soy bean (3) but not at 15 hours, except in very old plants. The results seem to show that growth of flower primordia in this plant can proceed on photoperiods longer than will permit their initiation.

The longest photoperiod at which abundant development of fruits occurred was 13 hours. The plants at this photoperiod resembled the 8 hour ones in their fruiting habit, but in the total amount of growth as measured by height and fresh weight they were more like the plants grown on longer photoperiods. The shortest photoperiod at which no flowering occurred even though flower primordia were known to be present at the beginning of the experiment was 16 hours.

II. Physiological and chemical studies

CULTURAL METHODS

The plants used in this experiment were grown out of doors in boxes of soil approximately 10 × 10 inches × 3½ feet. Abundant seeds were planted in each box so that when the plants were thinned to ten per box, 13 days after planting, a uniform stand was ob-

tained with little or no transplanting. In all, 272 boxes were planted. Of these, 192 were placed on trucks that could be moved into adjacent darkhouses at will and the remaining 80 were placed in a group on the ground near these cars. Mazda lights, controlled by electric time switches, were installed over the 80 boxes and also in the darkhouses. With this equipment it was possible to extend the photoperiod of natural light any desired amount. The lights were turned on shortly before sundown and off again about 2 hours later. The time the lights were turned off was changed as the season advanced, thereby maintaining a 16 hour daily photoperiod for the duration of the experiment.

The Mazda lights used were 100 watt each and were spaced sufficiently close so that 30-60 foot candles of light was supplied to the leaf surface. It has previously been shown that this intensity of light, used to extend a natural photoperiod beyond the critical length, is far in excess of that required to prevent flowering in the Biloxi soy bean (4).

The planting was made May 24 and all the plants were grown on 16 hour photoperiods until July 5. Beginning on this date, the 192 boxes of plants on trucks were given seven daily photoperiods of 8 hours each. The 80 boxes not on trucks were continued on 16 hour photoperiods. These plants served as long day controls, and from the time the seedlings emerged from the soil until the plants were finally harvested they never received less than a 16 hour daily photoperiod.

On July 12, after the plants on trucks had received seven daily photoperiods of 8 hours each, these plants were divided into three lots, each of which received a different photoperiod from that date until the end of the experiment. One lot was shifted to a 16 hour, another to a 13 hour, and the third was continued on an 8 hour photoperiod (table 5).

CHEMICAL METHODS

SAMPLING.—Samples of 60 plants each were drawn for chemical analysis July 5, 12, 19, 26, August 2; 9, and September 14. On July 5 a single sample was taken at random from the entire population. Since all the plants had received identical photoperiodic

treatments up to this time, additional samples were not needed. On July 12 two samples were selected at random, one from the 16 hour control plants and one from the thirty-two carloads that had received a week of 8 hour photoperiods. On each of the remaining sampling dates four separate samples were drawn, one from each of the lots shown in table 5.

All samples were taken at the beginning of the photoperiod of the date indicated. The beginning of the photoperiod of the various lots occurred at different times of the morning. This was advantageous since the sampling of one lot could be completed before time to har-

TABLE 5
SCHEDULE OF PHOTOPERIOD

DESIGNATION OF LOTS	PHOTOPERIOD RECEIVED AT VARIOUS TIMES		
	MAY 24- JULY 4	JULY 5- JULY 11	JULY 12 TO END OF EXPERIMENT
Controls			
16 hour.	16 hour	16	16
Transfers			
16 hour.	"	8	16
13 hour.	"	8	13
8 hour.	"	8	8

vest the next. The plants were cut at the cotyledonary nodes in all cases, and immediately brought to the laboratory. The fresh weight of the stems and the leaves was determined. The leaves were then cut into small segments and thoroughly mixed. From this a representative aliquot was withdrawn and dried in a forced draft oven at 70° C. The remaining tissue was ground in a food chopper. This grinding reduced the tissue to a very fine pulp, from which samples were drawn for analysis. The stems were treated in exactly the same manner as the leaves.

MOISTURE.—Samples consisting of 4–8 gm. of ground tissue were dried to constant weight in a vacuum oven at 80° C. under 2 to 3 cm. pressure.

SUGARS.—The sugars were determined by a method previously used (10). In brief the method is as follows: A sample of the ground

pulp was weighed into a counterpoised 200 ml. Kohlrausch sugar flask and covered immediately with sufficient boiling 95 per cent alcohol to give approximately 80 per cent alcohol covering the sample. The samples were brought to a boil on the steam bath soon after adding the alcohol and sufficient boiling water was added to give approximately 60 per cent alcohol. The extraction on the steam bath continued for 30 minutes. Preliminary tests with soy bean tissue showed that this time was sufficient. After the extraction, the flask was removed and made to volume with cold 95 per cent alcohol. The volume was again adjusted in the same manner just before making a determination. The reducing sugars were determined according to the Official Method (7), using the Munson and Walker gravimetric procedure. Total sugars were determined by the Official Method of acid hydrolysis. The hydrolysis was allowed to proceed overnight in a warm place. The amount of sucrose present was calculated as prescribed in the Official Method.

STARCH.—The starch was determined by a modification of a method previously reported (10). Samples of tissue that had been dried in a forced draft oven at 70° C. were ground with a Wiley mill to pass a 40 mesh sieve. Samples of this ground tissue, after redrying, were extracted free of sugars by means of a Soxhlet apparatus. The weight remaining after extraction was determined and the samples were reground to pass an 80 mesh sieve. Samples of this tissue were used to determine starch by a modification of the Official Method, in which an enzyme is used with subsequent acid hydrolysis. Fresh diluted saliva (1:10) was used as the enzyme for each digestion period, and enzymatic digestions were continued until microscopic examination showed no starch present. The samples were made to 250 ml. volume and filtered. Two hundred ml. of this filtrate was placed in a 250 ml. volumetric flask and treated with sufficient neutral lead acetate to precipitate interfering substances. The solution was made to volume and filtered. After deleading with anhydrous sodium oxalate, the samples were again filtered. A 200 ml. aliquot of this cleared solution was hydrolyzed 2.5 hours with 12.5 ml. of concentrated hydrochloric acid. Reducing sugars were determined by the Munson and Walker gravimetric procedure (7), and starch was calculated as described in the Official Method (7).

No attempt was made to determine dextrans, consequently any present were included with the starch.

TOTAL NITROGEN.—Samples used to determine moisture were transferred to Kjeldahl flasks and used for total nitrogen determinations. Since nitrates were present in all samples, the reduced iron method (11) was employed. Following the reductions, the Gunning method (7) was used to complete the digestion. Distillation was made into 0.1 N sulphuric acid and the excess acid was titrated with 0.1 N sodium hydroxide using the combination methylene blue-methyl red indicator.

EXTRACTION OF NITROGENOUS CONSTITUENTS.—A 50 gm. sample of the ground leaves or stems was transferred to a mortar and thoroughly ground with nitrogen-free quartz sand, with gradual addition of water to insure good grinding. After grinding for 10 minutes the suspension was decanted to a square of huck toweling suspended over a 2 liter beaker. After allowing to drain and then expressing by hand, the residue was returned to the mortar. The same extraction procedure was repeated two additional times. This resulted in complete removal of the non-protein nitrogenous constituents. The combined extracts were heated just to boiling, three drops of 10 per cent acetic acid and 7 ml. of dialized iron containing 5 per cent Fe_2O_3 were added. After boiling 2 minutes, the solution was filtered while hot and the residue in the Buchner funnel was thoroughly washed with hot water. The filtrate was cooled, made to 1000 ml. volume, and preserved with toluene. This extract contained only non-protein nitrogen. The procedure is essentially the same as that reported by STUART (5).

NON-PROTEIN NITROGEN.—Duplicate 100 ml. aliquots of the non-protein nitrogen extract were analyzed as described for Total Nitrogen.

ALPHA-AMINO-NITROGEN.—A 200 ml. aliquot of the non-protein nitrogen extract was treated as described by STUART (13) to remove interfering substances. After concentrating in vacuo to 50 ml., the amino-nitrogen was determined by the Van Slyke micro-apparatus.

AMMONIA NITROGEN.—The free ammonia was determined by the aeration method of SESSIONS and SHIVE (12), using duplicate 100 ml. aliquots.

NITRATE NITROGEN.—Nitrate nitrogen was determined on the ammonia-free samples by the method of SESSIONS and SHIVE.

SOLUBLE ORGANIC NITROGEN.—The difference between the soluble non-protein nitrogen and the sum of the ammonia and nitrate nitrogen was designated soluble organic nitrogen.

PROTEIN NITROGEN.—The difference between the total nitrogen and the soluble non-protein nitrogen was designated protein nitrogen.

EXPERIMENTAL RESULTS

On each sampling date, terminals of twenty-five plants from each sample were dissected. From these dissections, data were obtained as to the total number of nodes per plant and as to the kind of structures present at the growing tips. In those lots where flower buds were present, observations of their stage of development and measurements of their size were made. Height measurements from the cotyledonary scars to the terminals of twenty plants in each of the four lots were recorded at weekly intervals (fig. 1). The plants used for this purpose were numbered, so that measurements could be made each week from the same plants.

From these data it is seen that the 16 hour controls and the 16 hour transfers both continued to increase in length at a fairly constant rate throughout the season. The controls always maintained a position slightly in advance of the 16 hour transfers. The 13 and 8 hour transfers also increased in length at practically the same rate as the controls and 16 hour transfers until August 2. During the following week the rate decreased, however, and by August 16 elongation had ceased.

This behavior of growth in length is associated with the flowering and fruiting response made by the various lots. Although flower primordia were present on all of the plants in the three transfer groups, only the 8 and 13 hour transfers bloomed and set seed. Blooming began July 28 and rapid enlargement of pods began about August 9. The cessation of growth in length of the stems occurred at about the time the pods began to enlarge. On the other hand, the 16 hour transfers failed to form flowers and fruits, and it is seen that the elongation of their stems continued uninterrupted through-

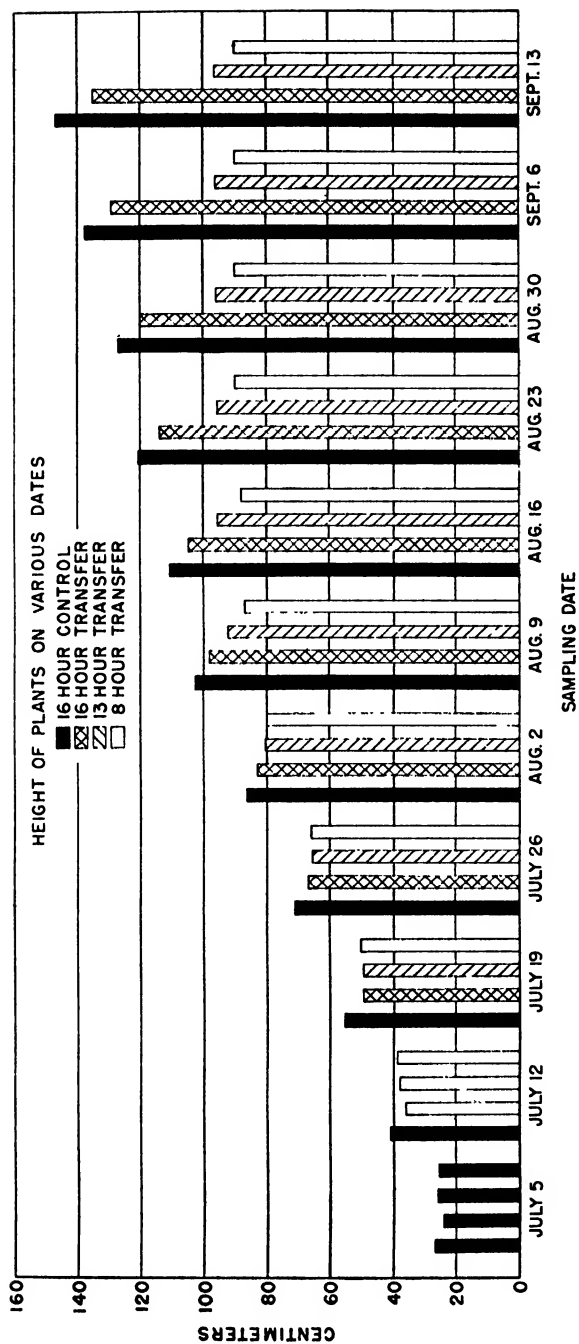


FIG. 1

out the season and at a rate only slightly less rapid than that of the 16 hour controls.

Differences in the fresh weight of plants of the various groups began to appear as early as July 26 and became accentuated as the season advanced (table 6). On September 14, when the final sample was taken, the 8 hour transfer plants were nearing maturity and had begun to lose some of their leaves. Their total fresh weight at this time was somewhat less than it was on August 9. Fresh weight of the 13 hour transfers was approximately the same as on August 9.

No marked differences in total dry weight per plant occurred in the various lots until about August 2. From this time the 16 hour controls gradually accumulated more total dry weight than any of the other lots. The 16 and 13 hour transfers went through the season with approximately the same accumulation of dry weight per plant. On September 14 the 16 hour transfers were slightly in advance and both lots were definitely heavier than the 8 hour transfers.

On the first sampling date, July 5, the plants had all received 16 hour photoperiods for 6 weeks, and were therefore alike in so far as photoperiod treatment was concerned. Growing points of twenty-five plants were dissected and no flower primordia were present. There was an average of 20.4 nodes in the main axis of each plant.

On July 12 two samples were drawn, one from the 16 hour controls and one from the plants that had received 8 hour photoperiods for one week. No significant difference was found in total nodes in the two groups; the 16 hour controls had 23.1 nodes per plant and the 8 hour plants 23.2. The most striking difference between the two lots was that flower primordia were present on the 8 hour plants and absent on the 16 hour controls. Twenty-five plants were examined in each group and no exceptions were found.

The flower primordia were easily recognizable with a binocular microscope but were in a very early stage of development. The bract at the base of the pedicel of each flower and the two bracts that are attached to the sides of the flower just below the calyx were well developed. The calyx was just starting to differentiate, even in the most advanced primordia.

On July 19 there were still no flower primordia present on the 16 hour control plants. The 8, 13, and 16 hour transfers all had flower

primordia present July 12 that were induced by the 8 hour photo-periods of the week previous. During the week of July 12-19, the development made by these was influenced by the length of photo-period to which they were subjected during that time. The buds on the 8 and 13 hour transfers were nearly three times the length of those on the 16 hour transfers. The mean lengths of twelve buds from comparable positions on plants of each of the three lots were 1.6, 1.7, and 0.6 mm. respectively for the 8, 13, and 16 hour transfers.

Aceto-carminic smears of the anthers of plants from the three groups were made. In the 8 and 13 hour transfers the microspore mother cells were full size and were separate from each other when pressed out of the anther. Early stages of meiotic division had not yet occurred. In the 16 hour transfers the anthers were very small and spore mother cells could not yet be recognized.

Although there were marked differences in the rate of growth of flower buds in certain of these lots during the week of July 12-19, there was a remarkable degree of uniformity in the number of new flower primordia initiated during that week. On July 12 flower buds were present in the buds of the main axis as high as node 19. A week later flower primordia were recognizable as high up the plant as node 23 in all three lots.

The average length of comparable flower buds on July 26 in the three transfer lots was 3.7, 3.9, and 0.7 mm., respectively. The 16 hour transfers had practically stopped development while the 8 and 13 hour lots had grown actively during the week. Pollen grains were present in the anthers of these two groups but no mother cells were yet visible in the 16 hour transfers. The controls were still without flower primordia.

On July 28 the first flowers opened on the 13 hour transfers. These were typical of flowers produced on soy beans under field conditions. The corollas of the 8 hour transfers on the other hand did not fully expand at anthesis. They pushed out beyond the calyx a short distance and remained in this condition for 2 or 3 days. The young pods then began elongation, indicating that pollination and fertilization had taken place. This behavior of soy beans grown on 8 hour days had been observed several times previously. Because of it the date of flowering cannot be determined in such plants. The

TABLE 6

PERCENTAGE MOISTURE AND DRY WEIGHT AND AVERAGE WEIGHT OF PLANTS RECEIVING VARIOUS PHOTOPERIODS

PHOTOPERIODIC TREATMENT	DATE SAMPLED	AGE OF PLANT AT TIME OF SAMPLING (DAYS)	PERCENTAGE OF MOISTURE		PERCENTAGE OF DRY WEIGHT				AVERAGE WEIGHT PER PLANT IN GRAMS									
			LEAVES		STEMS		FRESH					DRY						
			LEAVES	STEMS	LEAVES	STEMS	WHOLE PLANT	LEAVES	STEMS	PODS	WHOLE PLANT	LEAVES	STEMS	PODS				
Controls	July 5	42	81.54	82.55	18.46	17.45	33.78	24.36	9.42	6.13	4.49	1.64				
16 hour....	July 12	49	83.59	84.09	16.41	15.91	58.81	39.75	19.06	9.55	6.52	3.03				
Transfers																		
8 hour....			84.71	84.49	15.29	15.51	54.25	38.42	15.83	8.32	5.87	2.45				
Controls	July 19	56	84.14	83.25	15.86	16.74	93.68	63.96	29.72	15.12	10.14	4.98				
Transfers																		
16 hour....			83.60	83.31	16.40	16.69	84.31	58.10	26.21	13.90	9.53	4.37				
13 hour....			81.63	81.83	18.37	18.17	72.45	50.57	21.88	13.27	9.20	3.98				
8 hour....			84.58	83.58	15.42	16.42	83.95	58.48	25.47	13.20	9.02	4.18				
Controls	July 26	63	84.92	83.74	15.08	16.26	125.36	82.28	43.08	19.40	12.40	7.00				
Transfers																		
16 hour....			84.24	82.44	15.76	17.56	114.96	76.85	38.11	18.80	12.11	6.69				
13 hour....			81.57	81.89	18.43	17.11	99.78	67.68	32.10	17.96	12.47	5.49				
8 hour....			82.59	82.53	17.41	17.47	96.08	63.95	32.13	16.74	11.13	5.61				

Controls	Aug. 2	70	84.43	83.35	15.57	16.65	179.55	115.08	63.57	28.63	18.05	1058.
Transfers													
16 hour....			83.72	82.89	16.28	17.11	151.83	102.45	49.38	16.28	8.44
13 hour....			80.34	81.53	19.66	18.47	127.16	83.10	44.07	16.33	8.13
8 hour....			79.75	79.96	20.25	20.04	103.33	71.00	32.33	14.37	6.47
Controls													
16 hour....	Aug. 9	77	84.01	81.71	15.99	18.29	211.60	129.07	82.53	20.64	15.09
Transfers													
16 hour....			82.84	81.86	17.16	18.14	187.70	118.13	69.57	20.27	12.62
13 hour....			79.50	80.11	20.59	19.89	178.30*	103.87	57.55	16.88	21.29	11.45	1.51
8 hour....			78.24	78.91	21.76	21.09	149.75*	80.65	39.21	29.89	17.55	8.27	1.69
Controls													
16 hour....	Sept. 14	113	81.05	80.13	18.95	19.87	418.43	191.71	226.71	36.33	45.05
Transfers													
16 hour....			77.17	75.65	22.83	24.35	265.25	114.38	150.86	26.11	36.73
13 hour....			76.92	72.49	23.08	27.51	183.51*	40.20	55.41	81.90	10.66	15.24	27.51
8 hour....			76.92	73.63	23.08	26.37	114.96*	34.06	22.48	58.41	7.86	5.93	25.23

* Pods included.

fact that small pods made their appearance 3 or 4 days earlier than on the 13 hour transfers suggests that pollination and fertilization may have taken place slightly earlier in them than in the 13 hour transfers. The flower buds on the 16 hour transfers that were initiated during the induction period made no further development in most cases, but finally abscised. In a few plants flower buds on some of the lower branches continued a slow development throughout the season and finally produced a few open flowers in September. During the latter part of July and during August no new flower primordia were formed on the 16 hour transfers. On September 14, however, many plants had again begun initiation of flower primordia in the axils of leaves near the terminals of the main and side branches. Initiation of flowers in similar positions on a few of the controls was also observed at this time. When further dissections of plants of both lots were made September 28, new flower primordia were found near the tips of all plants. These primordia were formed in spite of the fact that the plants had been kept continuously on 16 hour photoperiods except for one week in the case of the 16 hour transfers. The tendency of old Biloxi soy bean plants to initiate flowers even when kept on long photoperiods, has been observed on several occasions.

The development of fruits and seeds on the 8 and 13 hour transfer plants proceeded normally, with the 8 hour plants somewhat in advance of the 13 hour ones throughout the remainder of the season. By August 9 the pods were 2-3 cm. long on both lots, and by September 14 the seeds were well formed but not yet mature.

CHEMICAL RESULTS.—The results of the chemical analyses have been calculated as percentage of both fresh and dry weight and as milligrams of a constituent per plant. The results as percentage of fresh weight are shown in tables 7 and 8, and the results as percentage of dry weight are shown in figures 2 to 17. Space does not permit the inclusion of the data calculated as milligrams of a constituent per plant.

NITROGEN.—The percentage of total nitrogen in the leaves and stems of a vegetative Biloxi soy bean decreased with the age of the plant. Most of this decrease occurred during the first 2 weeks of the experiment and was greater in the stems than in the leaves. When

plants were abruptly transferred to photoperiods of 8 hours, the percentage of total nitrogen in the leaves and stems increased and for several weeks remained higher than that of similar plants receiving a 16 hour photoperiod. Late in the season when pods were maturing on the 8 hour plants, the percentage of total nitrogen in both the leaves and the stems decreased to less than that of the controls. When other similar plants were transferred to 13 hour photoperiods after the week's induction, the total nitrogen on a dry weight basis immediately decreased in the leaves to a percentage similar to that of the control plants. This condition existed until the pods began to enlarge. At this time an increase in the percentage of total nitrogen occurred that was more pronounced in the leaves than in the stems. The stems of the plants receiving this photoperiod had approximately the same percentage of total nitrogen as the plants receiving an 8 hour photoperiod during the entire experiment. If the percentage of total nitrogen is considered from a fresh weight basis, however, these plants receiving a 13 hour photoperiod contained more nitrogen throughout the experiment than the controls, since they contained less moisture. On this basis the 13 hour transfer plants approached the 8 hour transfer plants in their percentage of nitrogen in both leaves and stems. The plants that were transferred to a 16 hour photoperiod, after the week of induction, had a higher percentage of total nitrogen in their leaves the first week following the transfer than the plants that had received a 16 hour photoperiod continuously. After that time the percentage of total nitrogen in the leaves of these closely approached that of the 16 hour control plants. The percentage of total nitrogen in the stems of the 16 hour transfer plants followed the same general trend as the leaves.

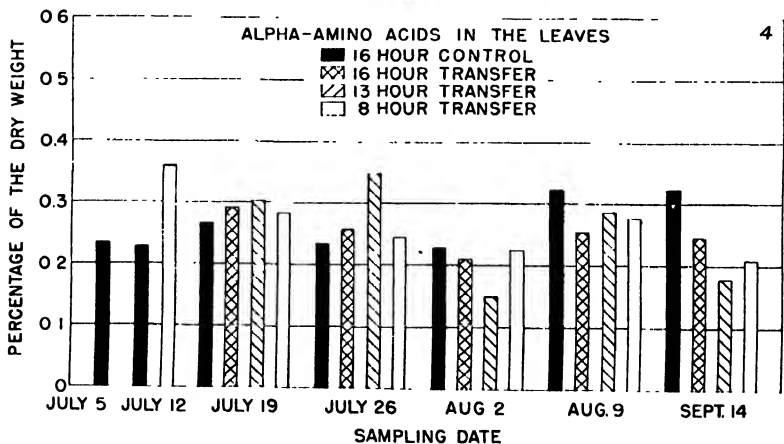
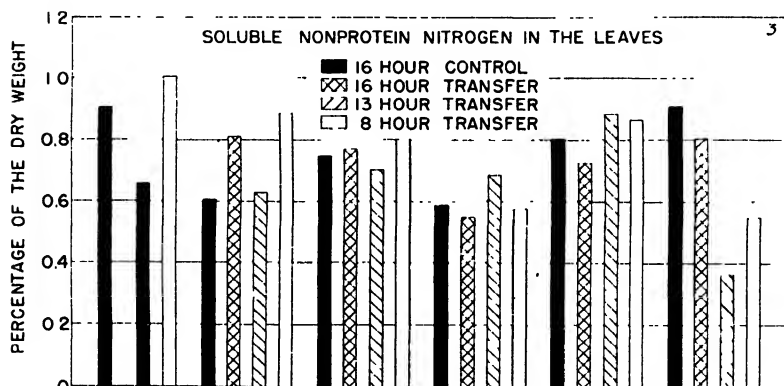
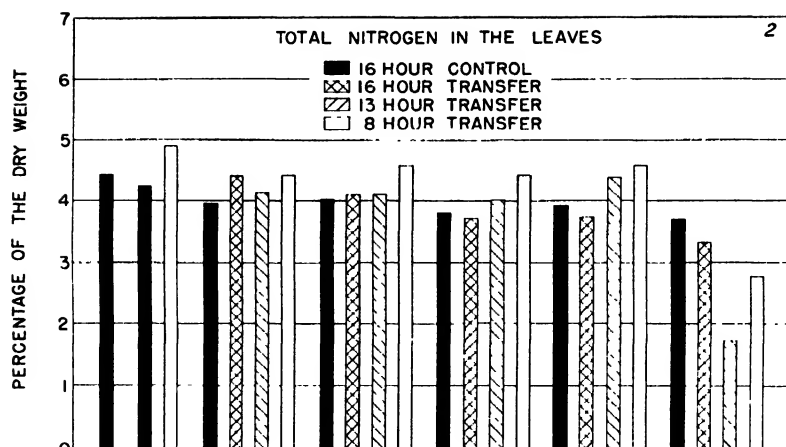
The actual milligrams of total nitrogen per plant increased as the plants became older. The differences between the milligrams of total nitrogen in the leaves of plants receiving a short photoperiod and those receiving a long photoperiod were slight during the induction period and until pods began to enlarge. As the pods matured the total nitrogen in the leaves and stems decreased. The stems of the plants receiving an 8 hour photoperiod contained less total nitrogen than those receiving a 16 hour photoperiod. This condition remained the same for the duration of the experiment.

TABLE 7

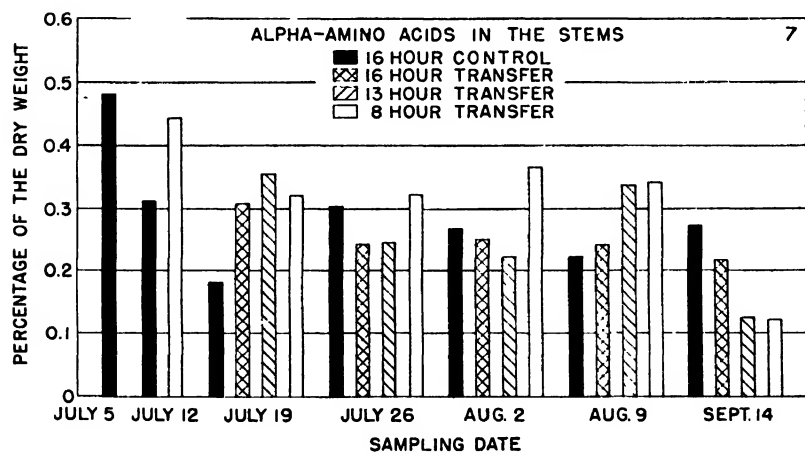
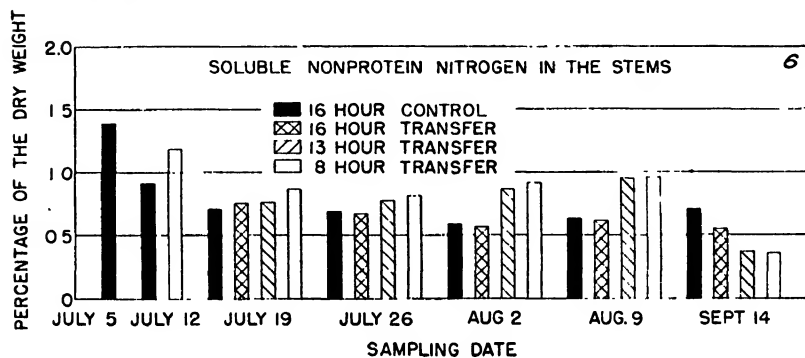
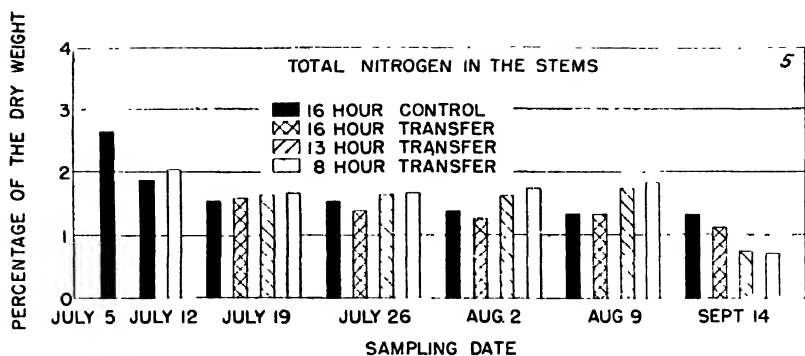
NITROGEN DISTRIBUTION IN LEAVES AND STEMS OF SOY BEANS SUBJECTED TO VARIOUS PHOTOPERIODS

PHOTOPERIODIC TREATMENT	DATE SAMPLED	AGE OF PLANT AT TIME OF SAM- PLING (DAYS)	PERCENTAGE OF NITROGEN BASED ON FRESH WEIGHT OF TISSUE													
			TOTAL NITROGEN		PROTEIN NITROGEN		SOLUBLE NON-PROTEIN NITROGEN		SOLUBLE ORGANIC NITROGEN		AMINO NITROGEN		AMMONIA NITROGEN		NITRATE NITROGEN	
			LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
Controls 16 hour....	July 5	42	0 826	0 458	0 659	0 215	0 167	0 243	0 108	0 156	0 043	0 084	0 003	0 005	0 056	0 082
Controls 16 hour...	July 12	49	0 702	0 300	0 594	0 155	0 108	0 145	0 085	0 114	0 037	0 050	0 005	0 006	0 018	0 025
Transfers 8 hour.....			0 755	0 319	0 597	0 136	0 158	0 183	0 114	0 152	0 055	0 069	0 005	0 013	0 039	0 018
Controls 16 hour....	July 19	56	0 635	0 254	0 539	0 135	0 096	0 119	0 083	0 099	0 042	0 030	0 003	0 003	0 010	0 017
Transfers 16 hour			0 728	0 310	0 595	0 161	0 133	0 149	0 107	0 120	0 047	0 051	0 003	0 002	0 023	0 027
13 hour.....			0 771	0 296	0 656	0 157	0 115	0 139	0 100	0 115	0 055	0 054	0 003	0 002	0 012	0 022
8 hour.....			0 690	0 275	0 553	0 133	0 137	0 143	0 114	0 118	0 044	0 053	0 005	0 004	0 018	0 021
Controls 16 hour.....	July 26	63	0 615	0 246	0 502	0 133	0 113	0 113	0 099	0 098	0 035	0 049	0 002	0 001	0 012	0 014
Transfers 16 hour.....			0 652	0 243	0 530	0 124	0 122	0 110	0 108	0 104	0 041	0 043	0 002	0 001	0 012	0 014
13 hour.....			0 763	0 279	0 633	0 147	0 130	0 132	0 120	0 119	0 064	0 042	0 002	0 001	0 008	0 012
8 hour.....			0 803	0 291	0 602	0 148	0 141	0 143	0 129	0 126	0 042	0 056	0 010	0 015	0 002	0 002

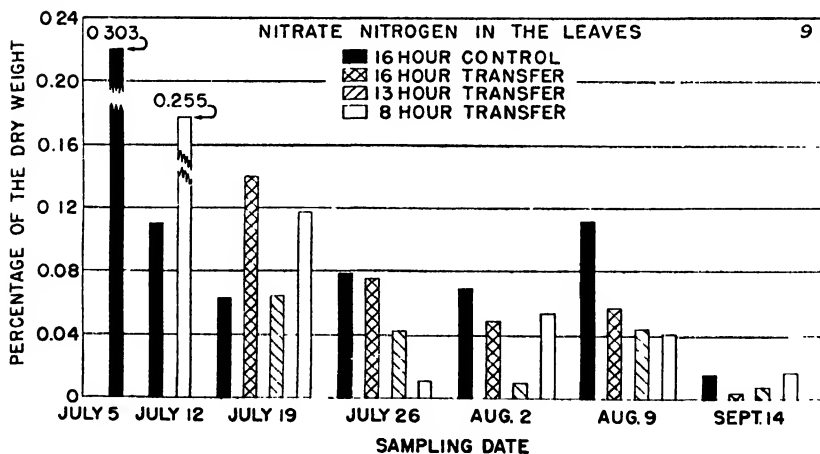
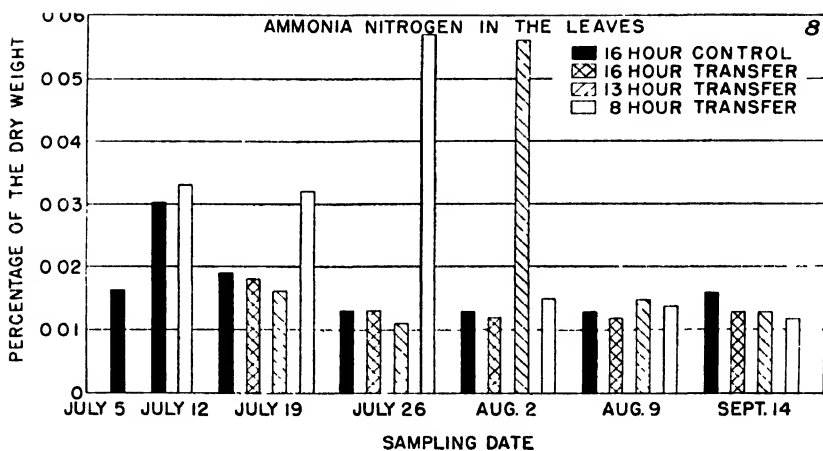
Controls	Aug. 2	70	0.599	0.223	0.507	0.124	0.092	0.099	0.079	0.083	0.035	0.045	0.002	0.002	0.011	0.014
Transfers																
16 hour.....			0.614	0.215	0.523	0.117	0.091	0.008	0.081	0.085	0.034	0.043	0.002	0.002	0.008	0.011
13 hour.....			0.801	0.297	0.666	0.137	0.135	0.160	0.122	0.131	0.039	0.041	0.011	0.025	0.002	0.004
8 hour.....			0.906	0.346	0.788	0.161	0.118	0.185	0.104	0.175	0.045	0.073	0.003	0.002	0.011	0.018
Controls	Aug. 9	77	0.633	0.241	0.503	0.124	0.130	0.117	0.110	0.097	0.051	0.041	0.002	0.002	0.018	0.018
Transfers																
16 hour.....			0.651	0.238	0.526	0.123	0.125	0.115	0.113	0.101	0.044	0.044	0.002	0.001	0.010	0.013
13 hour.....			0.908	0.347	0.726	0.156	0.182	0.191	0.170	0.177	0.039	0.067	0.003	0.002	0.009	0.012
8 hour.....			1.007	0.388	0.817	0.182	0.190	0.206	0.178	0.192	0.060	0.072	0.003	0.002	0.009	0.012
Controls	Sept. 14	113	0.711	0.261	0.537	0.121	0.174	0.140	0.168	0.134	0.061	0.054	0.003	0.002	0.003	0.004
Transfers																
16 hour.....			0.773	0.268	0.589	0.133	0.184	0.135	0.180	0.132	0.055	0.053	0.003	0.002	0.001	0.001
13 hour.....			0.413	0.203	0.326	0.100	0.087	0.103	0.082	0.099	0.041	0.035	0.003	0.002	0.002	0.002
8 hour.....			0.465	0.191	0.336	0.097	0.129	0.094	0.122	0.089	0.048	0.033	0.003	0.002	0.004	0.003



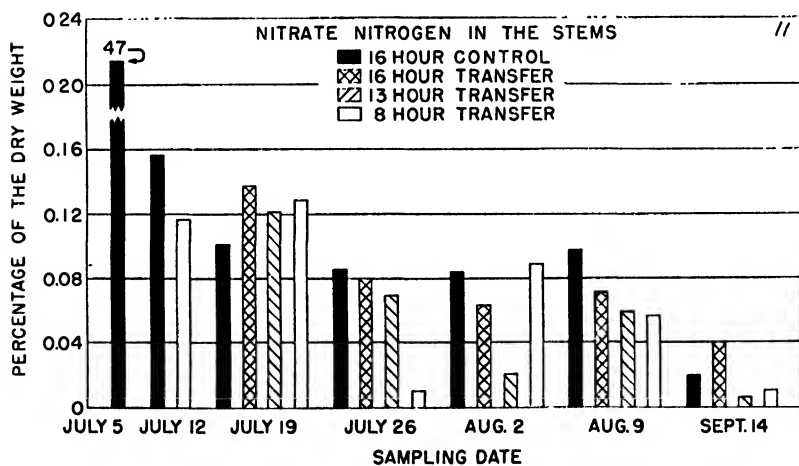
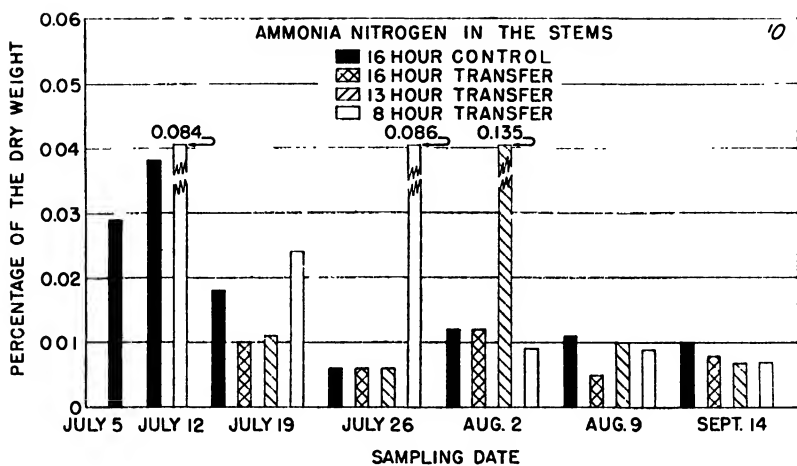
FIGS. 2-4



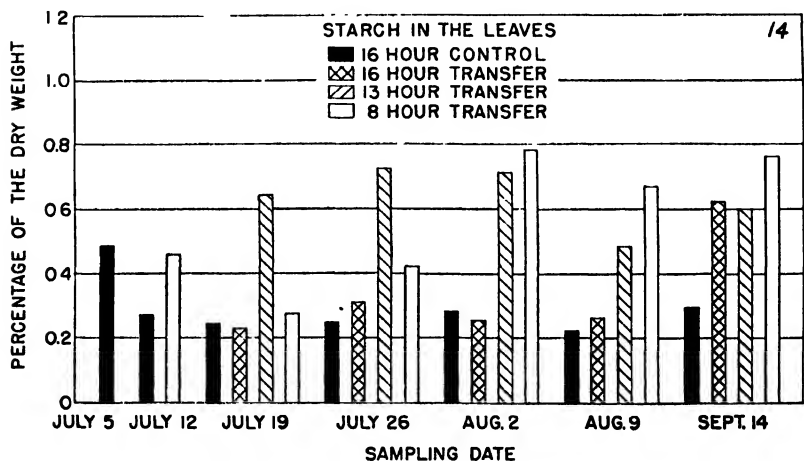
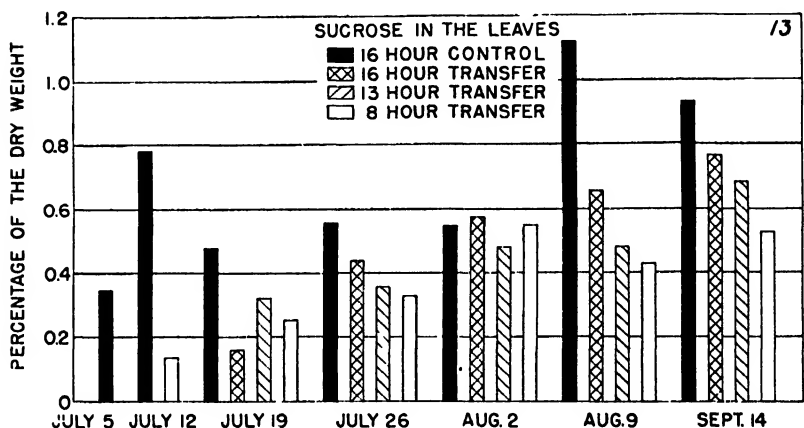
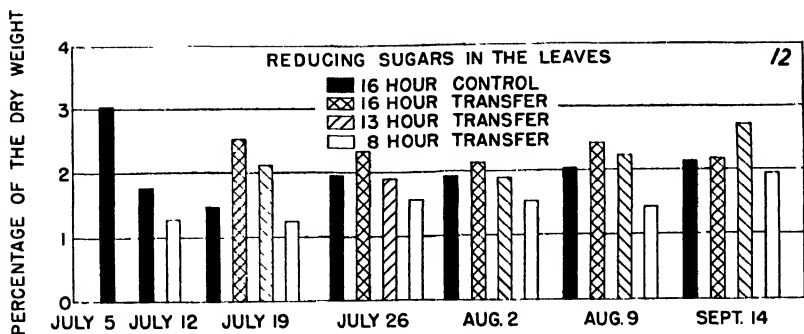
Figs. 5-7



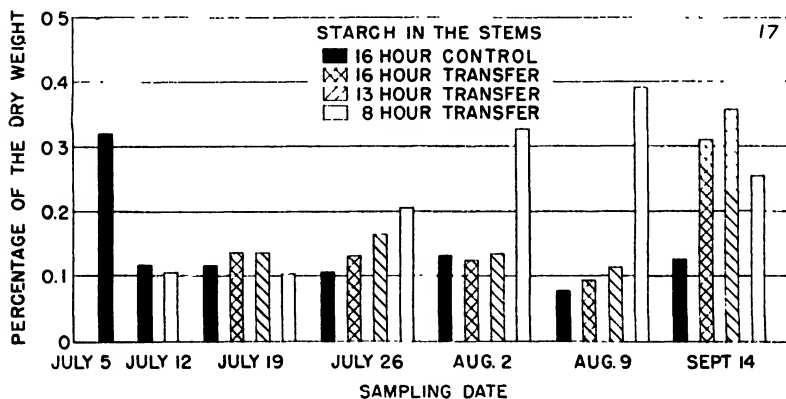
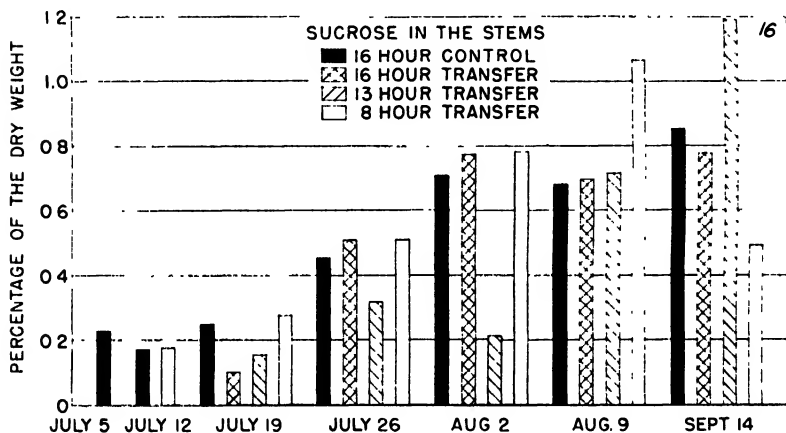
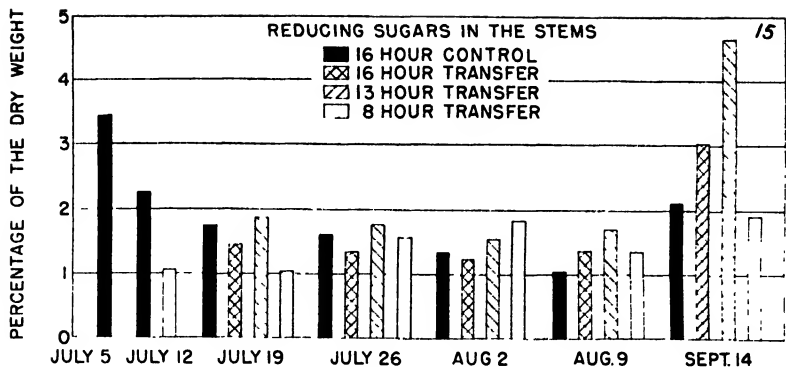
Figs. 8-9



FIGS. 10-11



FIGS. 12-14



FIGS. 15-17

During the induction period the percentage of the soluble non-protein nitrogen in the plants receiving an 8 hour photoperiod became greater than the controls in both leaves and stems. Those plants that remained on an 8 hour photoperiod following induction had a higher percentage of soluble non-protein nitrogen in the leaves on a fresh weight basis than the controls, until the time when pods were maturing. The percentage of soluble non-protein nitrogen in the leaves and stems of the 13 and 16 hour transfer plants was higher than that of the 16 hour controls and not greatly different from the 8 hour transfers, at the end of the first week following induction. Beginning with the second week after induction, the amounts of soluble non-protein nitrogen in the stems of 8 and 13 hour transfers approached each other and remained similar throughout the remainder of the experiment. The amounts of this constituent in the controls and 16 hour transfers showed a similar behavior. In the leaves on a dry weight basis greater fluctuations occurred, but at the time pods were beginning to enlarge there was more soluble non-protein nitrogen in the 8 and 13 hour transfer plants than in the plants of the other two lots. As the pods became mature the percentage of soluble non-protein nitrogen decreased rapidly in both the 8 and 13 hour transfer plants. The actual milligrams of soluble non-protein nitrogen in the leaves and stems was not greatly different in the various lots until the pods were nearing maturity on the 8 and 13 hour transfer plants. At this time the soluble non-protein nitrogen decreased greatly in both leaves and stems in these two lots. The alpha-amino acids in the leaves and stems of plants that received an 8 hour photoperiod were greater than those of the 16 hour control plants at the end of the induction period. After this there were fluctuations but these did not seem to have any general trend until pods were maturing on the 8 and 13 hour transfer lots. At this time there was a decrease of amino acids in both leaves and stems of these lots.

The percentage of ammonia nitrogen in the stems of the plants receiving 8 hour photoperiods was much higher than the controls after the induction period. In the leaves this condition occurred the following week. From that time until small pods began to appear the amount of ammonia in both the leaves and stems remained higher

TABLE 8

CARBOHYDRATE CONTENT OF LEAVES AND STEMS OF SOY BEANS
SUBJECTED TO VARIOUS PHOTOPERIODS

PHOTO- PERIODIC TREATMENT	DATE SAMPLED	AGE OF PLANT AT TIME OF SAM- PLING (DAYS)	PERCENTAGE OF CARBOHYDRATES BASED ON FRESH WEIGHT OF TISSUE							
			REDUCING SUGAR		TOTAL SUGAR		SUCROSE		STARCH	
			LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
Controls 16 hour..	July 5	42	0.558	0.603	0.652	0.674	0.064	0.040	0.089	0.056
Controls 16 hour..	July 12	49	0.295	0.355	0.449	0.406	0.128	0.028	0.044	0.018
Transfers 8 hour..			0.198	0.162	0.233	0.203	0.021	0.028	0.070	0.016
Controls 16 hour..	July 19	56	0.308	0.291	0.407	0.354	0.076	0.042	0.039	0.019
Transfers 16 hour..			0.418	0.286	0.464	0.319	0.026	0.017	0.037	0.016
13 hour..			0.392	0.339	0.476	0.383	0.059	0.027	0.118	0.024
8 hour..			0.195	0.168	0.248	0.226	0.039	0.045	0.042	0.016
Controls 16 hour..	July 26	63	0.296	0.261	0.403	0.355	0.084	0.074	0.038	0.017
Transfers 16 hour..			0.366	0.233	0.457	0.342	0.069	0.089	0.049	0.023
13 hour..			0.352	0.303	0.439	0.377	0.066	0.055	0.134	0.028
8 hour..			0.274	0.278	0.349	0.388	0.057	0.090	0.073	0.036
Controls 16 hour..	Aug. 2	70	0.302	0.222	0.411	0.362	0.085	0.118	0.044	0.021
Transfers 16 hour..			0.349	0.211	0.464	0.364	0.094	0.133	0.041	0.021
13 hour..			0.375	0.290	0.493	0.447	0.095	0.039	0.141	0.025
8 hour..			0.308	0.370	0.441	0.552	0.111	0.157	0.158	0.065
Controls 16 hour..	Aug. 9	77	0.324	0.187	0.532	0.331	0.179	0.124	0.036	0.014
Transfers 16 hour..			0.418	0.248	0.556	0.396	0.113	0.126	0.045	0.016
13 hour..			0.460	0.339	0.585	0.504	0.099	0.142	0.099	0.022
8 hour..			0.312	0.287	0.426	0.537	0.093	0.224	0.147	0.082
Controls 16 hour..	Sept. 14	113	0.408	0.421	0.618	0.618	0.177	0.169	0.055	0.025
Transfers 16 hour..			0.502	0.743	0.707	0.972	0.174	0.189	0.142	0.076
13 hour..			0.634	1.278	0.827	1.670	0.158	0.328	0.138	0.099
8 hour..			0.456	0.509	0.607	0.668	0.122	0.130	0.177	0.068

than in the controls. After pods had appeared, the percentage of ammonia in both leaves and stems was similar to that of the control plants. The plants transferred to 13 hour photoperiods contained approximately the same amount of ammonia in the leaves and stems as the controls, except at the time when very young pods were forming. At this time the percentage showed an abrupt rise over that of the controls. The ammonia nitrogen in the leaves and stems of the 16 hour transfer and the 16 hour control lots was similar throughout the experiment.

When the plants were subjected to a week of 8 hour photoperiod the nitrate nitrogen became greater in the leaves and less in the stem than control plants receiving a 16 hour photoperiod. When young pods were beginning to form on the 8 and 13 hour transfer lots, nitrate nitrogen was lower in the leaves and stems than in the controls. The times of occurrence of the low nitrate concentration corresponded to the time when ammonia was higher in these plants.

CARBOHYDRATES.—The results of the carbohydrate analyses are shown in table 8 and figures 12 to 17. At the end of the induction period the reducing sugars were lower in the leaves and stems of the plants receiving 8 hour photoperiods than in the controls. At the same time the amount of sucrose in the leaves was lower than that in the controls but remained the same in the stem. It was also evident from the data that the starch in the leaves during this period was higher in the short day than in the long day plants. In the stems the percentage of starch was very similar, regardless of photoperiod. Consequently the lower sucrose content of the leaves of plants on a short day may have been due to incomplete hydrolysis of available starch. During the week following the induction period, the leaves of the plants transferred to a 16 hour photoperiod contained more reducing sugars than those of any other treatment. The plants transferred to a 13 hour photoperiod had the next highest percentage of reducing sugars, and were followed closely by the 16 hour control plants. The least reducing sugar in any treatment was in the lots receiving the 8 hour photoperiod. The stems of the plants transferred to a 13 hour photoperiod contained the most reducing sugar after the first week of transfer, and the group of plants receiving the 8 hour photoperiods the least. At the same time more starch ac-

cumulated in the leaves of the plants receiving the 13 hour photoperiods than in any of the other treatments.

At the end of the second week after transferring the induced plants to different photoperiods, the percentage of reducing sugars was still high in the leaves of the 16 hour transfer group. The stems of the 13 hour photoperiod plants still contained the most sugars but the stems of the plants in all the treatments except the 8 hour photoperiod showed a decrease in the percentage of reducing sugars during the second week. The stems of the plants receiving an 8 hour photoperiod showed a marked increase in reducing sugars and sucrose and these plants were just beginning to form pods. This condition did not occur in the plants receiving a 13 hour photoperiod, however, and they were in practically the same morphological condition. Starch was still more abundant in the leaves of the 13 hour transfer plants than in any other group, while the stems of the plants on 8 hour photoperiods contained the most starch. Here again was a condition that occurred at the time of pod formation in the 8 hour photoperiod group but was not evident in the 13 hour photoperiod group.

At the termination of the third week after transferring the induced plants, small pods were present on the plants receiving the 8 and 13 hour photoperiods. These pods were too small to remove and were included with the stem sample. The pods on the plants receiving an 8 hour photoperiod were slightly more developed than those receiving the 13 hour photoperiods. This morphological condition was correlated with a high starch content in the leaves of both the 8 and 13 hour transfer groups. The stems of the plants receiving the 8 hour photoperiod also increased in starch.

The pods on the plants receiving 8 and 13 hour photoperiods were 1-3 cm. long the following week and were sampled separately. The leaves of the plants in these two groups contained much more starch than the leaves of the plants receiving 16 hour photoperiods. The stems of the plants receiving an 8 hour photoperiod were still much higher in starch than those receiving a 13 hour photoperiod, although both groups of plants were developing pods.

After 9 weeks in different photoperiods, the 8 and 13 hour groups were maturing seed, while the 16 hour transfer group had not yet flow-

ered. The results of the analyses of the leaves of the 8 and 13 hour groups showed that the milligrams of starch per plant had decreased during the production of seed. The stems of the plants receiving 8 hour photoperiods also decreased in starch but the stems of the 13 hour transfer plants increased in starch. The 16 hour transfer group contained much more starch in both leaves and stems than the 16 hour control plants. This was the first time that these two groups had shown any marked differences in starch content.

DISCUSSION

In this experiment an attempt has been made to establish two reference lines in so far as the chemical behavior of fruiting and vegetative soy beans is concerned. The plants for one of these have been maintained in a vegetative condition throughout the season by keeping them on long photoperiods. Flower primordia have been initiated on the plants of the other lot by transferring them to a short photoperiod of 8 hours and maintaining them on it throughout the remainder of the season. The composition of the two lots has been determined at frequent intervals. The other lots upon which flower primordia had been initiated in response to a week of 8 hour days were subjected to photoperiods just above and just below the critical, respectively, during the remainder of the season. The chemical composition of these was also determined on the same dates as the other two lots. Such a study permits a comparison of the influence of long and short photoperiods upon the metabolism of plants that already have flower primordia initiated. The plants in the group on photoperiods slightly below the critical became definitely similar to the 8 hour plants in several respects, while the plants on photoperiods above the critical more closely resembled the controls. In other characteristics such tendencies of these two lots were not found.

Both lots kept on photoperiods below the critical produced flowers and fruits. The other two lots remained without flowers until the last of September. Even then flowering was restricted to the 16 hour transfers, and occurred on only occasional plants. No fruits were formed.

The two lots kept on photoperiods longer than the critical in-

creased in length throughout the season. The other two lots ceased to elongate at the time pods began to form.

The total fresh weight per plant became somewhat greater in the two lots grown at photoperiods longer than the critical than in the others. This difference was brought about chiefly by the greater moisture content of these two lots. In absolute amounts of dry matter the differences were not great. The two lots transferred to 13 and 16 photoperiods, however, maintained a position intermediate to the 8 hour transfers and the 16 hour controls.

As to the chemical composition, the data show that the total nitrogen in both leaves and stems of the 16 hour transfers became similar to the controls, and the 13 hour transfers approached the 8 hour ones as the season advanced. Protein nitrogen showed a similar relationship in both leaves and stems after pods were beginning to expand. The soluble organic nitrogen fraction showed similar difference in the stems but not so much in the leaves. The differences in this fraction in the stems were apparently due to compounds other than alpha-amino acids, since they did not show such a relationship until late in the season. The two lots of plants that fruited had a sharp but temporary increase in ammonia immediately after fruits began to form; the two non-fruited lots did not have this sharp fluctuation. Simultaneously with the increase in ammonia the nitrates in the two fruited lots were low.

With respect to their carbohydrate content, the two intermediate lots, which received the 13 and 16 hour photoperiod after induction, did not tend to become associated in composition with their respective reference groups as much as they did in respect to their nitrogen compounds. There was, however, a rather direct relationship among the three transfer groups in the reducing sugar content of the leaves and the length of the photoperiod. In the stems, on the other hand, the 13 hour transfers were higher than either of the other two transfer groups. The starch content of the leaves in the 8 hour and 13 hour groups and in the 16 hour transfers and control plants became similar after pods began to form, the former groups becoming much higher in starch than the latter groups. In considering these relationships it is necessary to bear in mind that the growth rates of the two lots receiving a 16 hour photoperiod were approximately

the same, and that they received the same number of hours of light and dark. The reserve carbohydrates were approximately the same in these two groups. The simple sugars were always higher in the leaves, however, and until later in the season lower in the stems of the plants that had received a week of short photoperiod. The reasons for this apparent difference in translocation cannot be explained from the data available. The growth of the 8 and 13 hour transfer plants was inhibited by their respective photoperiods, but these plants received different amounts of light for photosynthesis during the day and the following period of darkness was longer in the 8 hour than in the 13 hour group. Consequently the plants in the 13 hour transfer group always contained more reducing sugars in their leaves and stems than the 8 hour transfer plants. This relationship was also true of the reserve carbohydrates until pods began to enlarge; then the 8 hour plants had more starch than the 13 hour plants. This was probably due to the fact that the 13 hour plants set more fruit and as a consequence utilized more of the starch in their growth and development.

MURNEEK (8), working with Biloxi soy beans, reported that a high C/N ratio was not evident until the pods were maturing. He concluded that while a high C/N ratio was probably essential to normal fruit development, induction of flower primordia occurred when the ratio was lower than that of vegetative plants. In our work a higher percentage of nitrogen and a lower percentage of soluble carbohydrates occurred in the plants following the induction of flower primordia. There was an increase of starch in the leaves at this time. However, these differences in composition do not seem to have any direct causal relation to induction.

Following the induction period, the total carbohydrate and total nitrogen ratio in the leaves of the 16 and 13 hour transfers paralleled the 16 hour control plants while the ratio was lower in the 8 hour transfers. This ratio in the leaves of the plants bearing fruit was higher than the control only when the pods were maturing. The ratio of starch to total nitrogen was always higher in the leaves of the 13 hour transfers than in the controls, but the ratio in the 8

hour transfers did not become higher until pods were beginning to enlarge. The relationship of carbohydrates to nitrogen did not vary greatly until pods were either forming or maturing.

Summary

1. Biloxi soy bean plants with flower primordia initiated upon them were transferred to photoperiods of 8, 10, 12, 13, 14, 15, 16, and 18 hours. The development of these primordia and the flowering and fruiting responses of the plants were determined.

2. The plants transferred to photoperiods of 8 to 13 hours bloomed nearly simultaneously and all produced fruits. The yield of fruits on the 8 hour lot was somewhat less than on the 10, 12, and 13 hour lots.

3. Flowering on the 14 and 15 hour plants was later than on those of shorter photoperiod, the flowers were less numerous, and no fruits were formed.

4. No flowers opened on the 16 and 18 hour plants during the experiment.

5. When plants were transferred to a range of photoperiods after flower primordia were initiated it was found that the longest photoperiod on which fruit formation occurred was 13 hours and the shortest one on which no flowering took place was 16 hours.

6. Plants were grown for biochemical studies on 8, 13, and 16 hour photoperiods after initiation of flower primordia. A control lot was grown continuously on 16 hour photoperiods. The carbohydrate and nitrogen metabolism of these plants was determined at frequent intervals throughout the season.

7. At the end of the week's induction period the total nitrogen and soluble non-protein nitrogen were higher in the plants receiving 8 hour photoperiods than in the controls. Carbohydrates were lower than in the controls, with the exception of starch in the leaves which was higher.

8. The total nitrogen in both leaves and stems of the 16 hour transfers became similar to that of the controls and the 13 hour transfers approached the 8 hour ones as the season advanced. The soluble non-protein nitrogen showed the same relationship in the

stems. There was an abrupt rise in the amount of ammonia in the leaves and stems of the 8 and 13 hour transfer plants when pods were just beginning to form.

9. The amount of soluble carbohydrates in the transfer groups seems to be correlated with the length of photoperiod. Starch accumulated in the leaves and stems of the 8 and 13 hour transfers when pods were beginning to form.

10. Two groups of plants with flower buds initiated upon them and subsequently grown at photoperiods just above and just below the critical showed progressive deviation from each other in their carbohydrate and nitrogen metabolism. Those grown below the critical became similar to plants grown on 8 hour photoperiod, while those above the critical became similar to plants that had been kept vegetative by growing them continuously on 16 hour photoperiod.

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CURRENT LITERATURE

Submikroskopische Morphologie des Protoplasmas und seiner Derivate (Submicroscopic Morphology of Protoplasm and Its Derivatives). By A. FREY-WYSSLINGH. Protoplasma Monographien 15. Berlin: Borntraeger, 1938. Pp. 317. Figs. 138.

The author has set out upon the ambitious task of introducing the "average biologist" to the field of physics and biochemistry pertaining to cellular structure. Methods and terminology are given in the first hundred pages; the second hundred are devoted to the fine structure of cytoplasm, nucleus, and chloroplasts; the last hundred pages to the derivatives. Although the author is a plant physiologist, the third part of the book contains chapters on chitin, silk, keratin, tendons, muscle fibers and nerves. Cellulose, cutin, and starch grains receive equal attention.

The style of writing is fluent and often intriguing, and the many diagrams are without exception clear and illustrative.

The science of submicroscopic structures is concerned with the order of magnitude below 0.1μ and above $1A$, and it stands between cytology and the science of molecular structure. With new diagrams of old facts the reader learns or remembers about phases and phase-changes, molecular structure and molecular models. This prepares for a non-exhausting but to many probably instructive discussion of monolayers, liquid crystals, and myelin figures. There follows an important chapter on micellar science, which describes polymerization, especially of polysaccharides, chain-lengths, structural viscosity, contact points (Haftpunkte), and external micellar properties. Optical and x-ray methods are described, although the author's own field is primarily the former. "The micellar condition of a gel structure differs from the dispersoid in the fact that the constituent units can not be completely solvatised but remain attached at certain points of contact. With the rupture of these contact-points the micellar character is lost."

In the chapter on cytoplasm, polypeptide chains with side grouping showing various properties are described. Contact points derived from polypeptide chains may be of four kinds: (1) homopolar cohesions (mutual attraction of lipid groups); (2) heteropolar cohesions (attractions of groups with dipole effect); (3) heteropolar valence bindings as salt or ester formation; and (4) homopolar valence bindings or bridge formation (S-S). Each type of binding is extensively discussed. pH and rH are discussed and compared as they affect micellar structure. There is a helpful discussion on fixation. "It is a fundamental difference between living and dead gels that in the former contact points are continuously under reconstruction." Perhaps more could have been said

on permeability, but the author offers a diagram; and following MEYER, a general formula is presented which takes into simultaneous consideration ion-mobility and lipid solubility. Genes, chromosomes, and the spindle are discussed tentatively. A very searching treatment presents the author's most original fields, the chloroplasts and the cell walls built of cellulose, as well as the cutinized ones. The structure of starch grains is considered in the last chapter on reserve materials. In many cases the author has consciously over-simplified the problems, and few will have his identical approach to biology; but he has made it exceedingly easy for the reader to gain introduction at least, where previously only the specialist was admitted.—B. R. NEBEL.

The American Species of Crepis; Their Interrelationships and Distribution as Affected by Polyploidy and Apomixis. By F. B. BABCOCK and G. L. STEBBINS, JR. Carnegie Institution of Washington Publication No. 504, 1938. Pp. iv+199. Illustrated.

This is another of the recent striking examples of the integration of the findings of morphology, cytogenetics, ecology, and geographical distribution in a lucid and rational treatment of a difficult taxonomic complex, and in the formulation of plausible hypotheses on the origin and spread of the species and forms.

Seven adventive and twelve indigenous species of *Crepis* are recognized in western North America. The latter fall into three groups of 2, 1, and 9 species. *C. nana* and *C. elegans* in the first group are arctic-alpine forms of old world affinity ($x = 7$). The polymorphic but exclusively diploid *C. runcinata* ($x = 11$) forms the second, a wide-ranging species centering in the central Rocky Mts. The other nine species are best developed in the Columbia Plateau and northern Great Basin on arid, well drained hill and mountain slopes. These species are based on seven morphologically distinct and genetically isolated diploid forms ($2n = 22$) of somewhat restricted range, centering in northeastern California and adjacent Oregon, and central Washington, each occupying a particular habitat. These seven diploids are connected morphologically and geographically by a series of auto- and allopolyploid forms ($2n = 33, 44, 55, 77, 88$), generally apomictic. The presumed allopolyploids show their hybrid origin by combining the morphological characteristics and ecological tolerances of the presumed ancestry.

In the taxonomic treatment, the polyploid agamic complex caused the greatest difficulty. The authors chose an intermediate course between recognizing the entire complex as a single species and assigning specific rank to each apomict. Each diploid was taken to represent the morphological type of a species. "Those apomicts which fall (except for quantitative differences) within the range of variation of the diploids, as well as those partial allopolyploids which, by the possession of certain well-marked characteristics, show their undoubted affinity to one or other of the diploids, are assigned to the original species which they resemble. The other allopolyploid apomicts are grouped into agamospecies, each of which has approximately the same degree of variation

as the original species, and contains apomicts which appear to have originated from the same or similar hybridization."

"Within the species, the diploid form is carefully described and distinguished from the polyploids, and in addition the species is divided into subspecies or varieties on the same basis as are sexual species The delimiting of species and subspecies has been done more or less independently of the nomenclatural history of the forms involved." Though the numerous apomicts have no taxonomic status, some of them have been named and described in the text under the species to which they were assigned. In addition, tables showing the characteristics in which they more closely resemble other diploids have been given for the named apomicts of each species.

The resulting treatment appears to be one which can be used satisfactorily by persons of differing botanical acumen, from those wishing only to assign a specific name to a plant in hand to those interested in the finest correlations of genetic constitution and environment.—C. E. OLMSTED.

Introduction to the Botany of Field Crops. Vol. I, *Cereals*; Vol. II, *Non-cereals*.

By J. M. HECTOR. Johannesburg: Central News Agency, Ltd., 1936-1938. Pp. lxxv+1127. Figs. 448. £3.10 per set.

In two volumes the author has assembled a wealth of material on a wide variety of economic plants, usually classed as field crops. The first volume is devoted to the cereals; first a chapter treating of them in general, then successive chapters dealing in detail with oats, wheats, rye, barleys, rice, millets, sugar canes, sorghums, and maize. Other grasses are considered very briefly if at all. The second volume deals with the Liliaceae, Moraceae, Polygonaceae, Chenopodiaceae, Cruciferae, Leguminosae, Linaceae, Malvaceae, Umbelliferae, Convolvulaceae, Solanaceae, Cucurbitaceae, and Compositae. Under each family there is a key to the several genera which contain species of economic importance, and at the end a comprehensive bibliography.

The treatment throughout is predominantly from a systematic and structural viewpoint, although a few details of ecological or physiological significance are added. In so far as material has been available for compilation, the entire plant has been considered—anatomically, histologically, and cytologically. The illustrations are many, mostly excellent but variable in this respect, partly owing to the quality of the original illustrations which have been copied and partly to the methods used in reproducing others. But the breadth of concept and outlook of this excellent work far outweigh any minor defects of illustration or typography. The author's thesis, as stated in the introduction, holds that a knowledge of form and structure of a plant enables the student better to understand its life processes; that the ecological viewpoint connects with the physiological. Familiarity with the reactions of a plant to its environment forms the groundwork for the study of crop ecology, a study which will pave the way to the development of an "adjusted agriculture." It is not too much to hope that such a goal may be attained, and these volumes constitute an effective aid toward such accomplishment.—K. C. HAMNER.

Native Woody Plants of the United States. By WILLIAM R. VAN DERSAL. Washington: Government Printing Office, 1938. Pp. 362. \$1.75.

An indexed list of several hundred woody plants growing in the United States, naming each species as unmistakably as possible, and giving all available data pertinent to its use in erosion control and wildlife conservation. The United States is apportioned into thirty-two growth regions which are briefly characterized. The maps show respectively the plant growth regions of the United States, the climatic regions of Thornthwaite superimposed on Mulford's plant growth regions, and the important soil regions superimposed on the same growth regions. There are a number of good informative illustrations, an extensive bibliography, and a list of the common and scientific names of woody plants.—E. J. KRAUS.

Index to North American Ferns. By MAURICE BROWN. Orleans, Mass.: Published by the compiler, 1938. Pp. 217.

For more than three decades the study of North American ferns and their allies has increased the knowledge of habitats and ranges, created changes in nomenclature, and suggested rearrangements of genera and species to fit present day phylogenetic concepts. This Index is the first successful effort to assemble such information for use of pteridological amateurs as well as specialists. The book consists of a list of pteridophytes of North America (north of Mexico) arranged alphabetically by genera. Etymology and synonymy of names and the habitats and ranges of species are concisely given. The distribution of the species is so stated that the direction of its migration is evident. A systematic outline of the North American pteridophytes, a tabulation giving the number of introduced and native species (also forms and varieties), an author list, and an index complete enough to include synonyms add to the usefulness of the book.—P. D. VOTH.

La Distribution géographique des végétaux dans la région méditerranéenne française. By CH. FLAHAULT. Paris: Paul Lechevalier, 1937. Pp. xi+178. Maps.

Das Pflanzenleben der Ostalpen. By RUDOLPH SCHARFETTER. Vienna: Franz Deuticke, 1938. Pp. xvi+419. Figs. 73. Map.

A critical comparison of these two books indicates much of the development of geobotany in the last 40 years, including the elaboration of new concepts and the pulverization and refinement of old ones. The first volume has been printed from an unpublished manuscript of the late Professor FLAHAULT, which was awarded the *Prix Gay* in 1897. This manuscript and accompanying maps summarized in unified and simple fashion the author's own observations and researches of the preceding 16 years on the vegetation of the French Mediterranean region, corresponding in general to the range of *Quercus ilex* or its associated species in France. The editor, H. GAUSSEN, assures us that no other complete description of this region has ever appeared. In contrast, SCHARFET-

TER's work is based upon the compilation of data from over 1000 references, and his observations during 30 years. His area is bounded by the Danube and the Po on the north and south respectively, a line running through the Lake of Constance and Lake Como on the west, and an irregular one from Pressburg through Agram and Laibach to Trieste on the east. The treatment is decidedly technical. The book is therefore of value only to persons familiar with the concepts, terminology, and some of the facts of central European geobotany, for whom it does serve as a not-too-well digested record and summary of the past researches on the region, and as a basis for future investigation. Valuable maps of the important communities of each region are found in the respective works. — C. E. OLMSTED.

An Introduction to Botany. By A. W. HAUPT. New York: McGraw-Hill Book Co., 1938. Pp. xii+396. Figs. 278.

HAUPT's definition of botany as "the science of plant life" indicates the scope of this book, the outstanding feature of which is its excellent correlation of structure and function. Since a knowledge of structure is necessary for an understanding of all other aspects of botany, morphology is emphasized; but stress is laid upon the concept of the plant as a living thing. The volume is intended to cover a semester's work; but it should serve equally well for a three months' course, especially a summer course, where so many of the students are teachers. The second part, with its evolutionary sequence, is particularly flexible and would be useful where a whole year can be devoted to the subject.

The illustrations are particularly good, most of them being prepared by the author himself.—C. J. CHAMBERLAIN.

Research and Statistical Methodology, Books and Reviews, 1933-1938. OSCAR KRISEN BUROS, Editor. New Brunswick: Rutgers University Press, 1938. Pp. vi+100. \$1.25.

An assemblage of announcements, titles, and reviews of books on statistical methodology issued during the past five years. A work such as this is very useful, both because it presents the viewpoint and criticisms of several reviewers of any particular book, and because it brings these together in a single place where they are readily accessible instead of remaining widely scattered among numerous journals in different fields.—E. J. KRAUS.

Common British Grasses and Legumes. By J. O. THOMAS and L. J. DAVIES. London: Longmans, Green & Co., 1939. Pp. vii+124. \$2.20.

While devoted more especially to the common grasses and legumes with which the British farmer is concerned, there is much in this book that is helpful to agronomists in many other countries, because of the wide distribution of many of the species listed. Workable keys to the vegetative characters of some common grasses and legumes, and clear cut and meaningful illustrations, make this brief volume readable and helpful.—K. C. HAMNER.

THE BOTANICAL GAZETTE

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PHLOEM OF WHITE PINE AND OTHER CONIFEROUS SPECIES

LUCY B. ABBE AND A. S. CRAFTS

(WITH FORTY-SEVEN FIGURES)

Introduction

The phloem of the gymnosperms differs in many details from that of the angiosperms. Certain structural features of the long fusiform elements found in conifers do not fit the classical picture of the sieve tube as a perforate element, adapted to rapid longitudinal conduction. While plasmodesmata in the sieve plates of many monocotyledons and certain herbaceous dicotyledons are extremely fine, approximating in diameter those of the primary pit areas of phloem and cortical parenchyma, the protoplasmic connections of the gymnosperms are even more tenuous. It is difficult to reconcile their structure with the concept of tubular connections developed from studies on vines and other specialized angiosperms. A detailed description of the phloem of white pine, *Pinus strobus* L., and of other coniferous species should clarify the structural relation of these plasmodesmata to their role in conduction. In addition, it should provide information of general morphological interest.

Material and methods

In addition to *Pinus strobus*, which was used in developmental studies, the following species were investigated:

Abietineae	Cupressineae
<i>Pseudotsuga taxifolia</i>	<i>Thuja plicata</i>
<i>Tsuga canadensis</i>	<i>Juniperus virginiana</i>
<i>Picea glauca</i>	<i>Chamaecyparis obtusa</i>
<i>Picea canadensis</i>	
<i>Abies concolor</i>	Araucarineae
<i>Pinus rigida</i>	<i>Araucaria</i> sp.
<i>Pinus densiflora</i>	<i>Agathis</i> sp.
<i>Pinus parviflora</i>	
<i>Pinus resinosa</i>	
<i>Pinus monticola</i>	
Taxodineae	Taxineae
<i>Taxodium distichum</i>	<i>Taxus canadensis</i>
<i>Sequoia gigantea</i>	<i>Taxus cuspidata</i>
<i>Sequoia sempervirens</i>	

Differentiating and functioning phloem tissues are highly hydrated and hence soft and plastic. To avoid shrinkage and distortion resulting from the use of alcohol and drastic killing agents, living tissue was examined wherever possible. In removing the cambium and phloem from a tree and in sectioning and studying the living tissue, the methods of BAILEY (2) and of BAILEY and ZIRKLE (3) were followed.

For studies of sieve tube contents, tangential or radial sections 30-100 μ thick were cut from blocks of living tissue. Owing to the transparency of the untreated cambium and young phloem, details of structure within the sieve tubes may be seen, since living cells often occur close to the surface of the section. Cambial initials and young sieve elements in such sections appear to be but slightly injured, if at all, the cytoplasm continuing to stream normally for from several hours to many days, depending on external environmental factors. In many cases the sections of white pine were cut and placed under observation within 15 minutes after removal of the tissue from the living tree.

During 1932, blocks of white pine were collected at intervals of two or three weeks throughout the year, and the extent of callus development in the sieve pits was studied both before and after staining with water soluble anilin blue. These results were checked

during 1933 and part of the growing season of 1935. The nature of the sieve tube contents was studied both in unstained cells and after vital staining of the vacuoles. Plasmolysis tests were conducted on living sections stained with neutral red. Following examination in the living condition, many of the sections were killed in IKI solution, swelled in IKI in 5 per cent sulphuric acid, stained with water soluble anilin blue, and mounted in a medium containing glycerin and zinc chloride (5). For sieve pit structure, dilute neutral red followed by NEBEL's (20) callus stain was also used.

For permanent records, small blocks of the outer wood, cambium, and inner bark were killed in Flemming's strong fixative or in boiling water, and sectioned after imbedding in paraffin by the butyl alcohol method. Although useful as a record, such sections, in comparison with the untreated fresh material, present only a caricature of the living cambium and of sieve tube contents and walls.

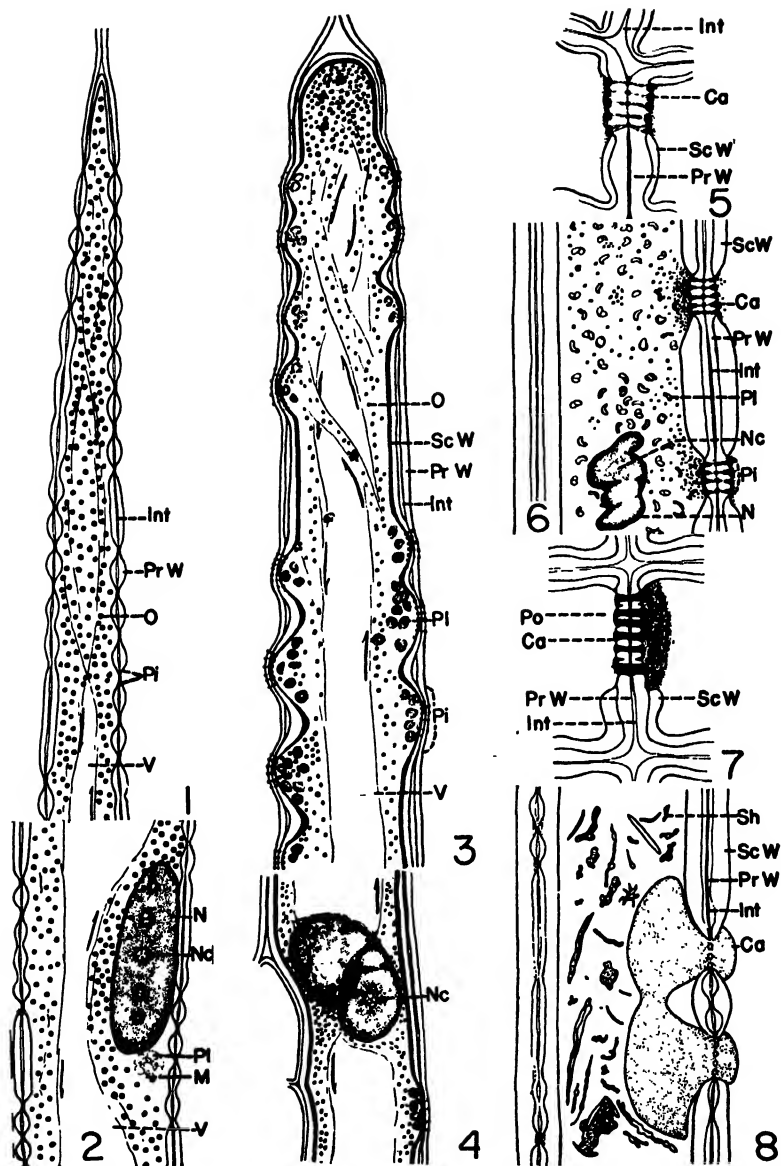
Observations

PHLOEM ONTOGENY IN WHITE PINE

INITIATION OF SIEVE TUBES.—Sieve tubes are of cambiform outline in tangential sections and, unlike tracheids, undergo little or no additional lengthening at the tips from the time of their derivation from the cambium to maturity. In transverse section they are usually more or less rectangular (fig. 9), having a tangential diameter approximately that of the cambial initials. The radial diameter is several times that of the initials. The length of cambial initials and sieve tubes is about one hundred times their tangential diameter (2, p. 659, fig. 15).

The wall of the cambial initial of white pine has been described and figured by KERR and BAILEY (17). There are essentially three layers present, two primary walls containing cellulose as well as polyuronides, and an intercellular substance composed of polyuronides. Numerous primordial pits occur in the radial walls of these initials (fig. 1).

The contents of the cambial initials have previously been described and figured (1, 2). The streaming cytoplasm of the cambial initial commonly contains a single large vacuole. In the winter numerous conspicuous oil globules (fig. 10) are present in the slowly



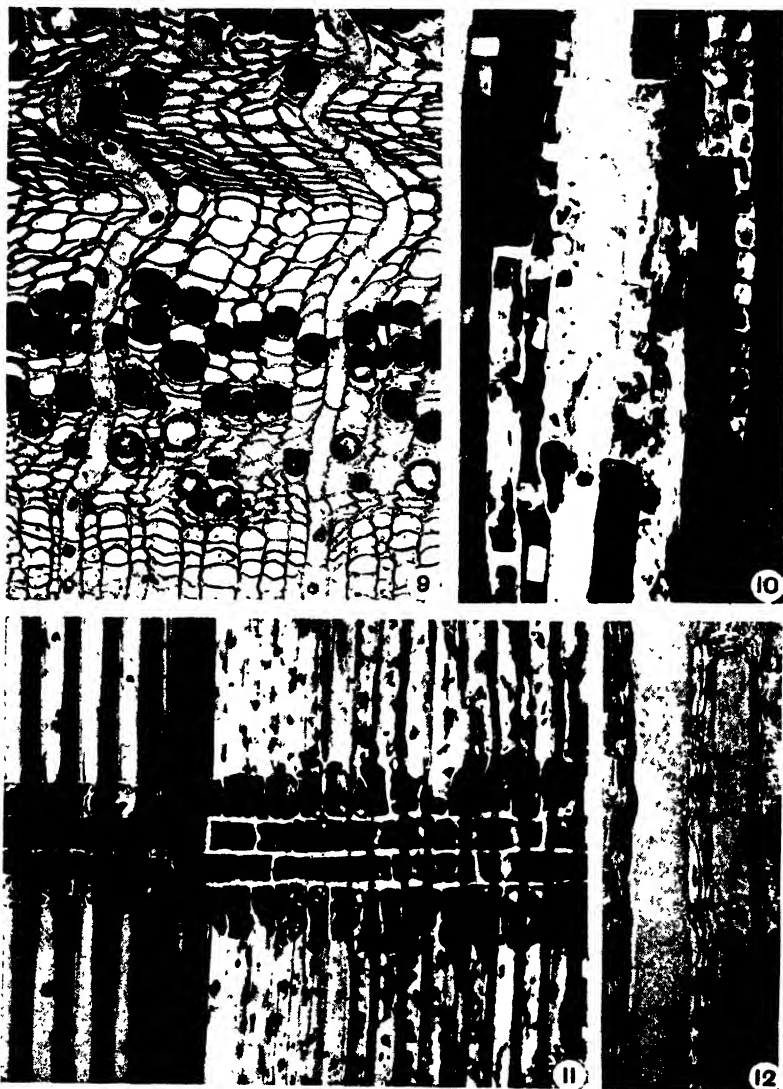
FIGS. 1-8.—White pine. (*Ca*, callus; *Int*, intercellular substance; *M*, mitochondria; *N*, nucleus; *Nc*, nucleolus; *O*, oil globule; *Pi*, pit; *Pl*, plastid; *Po*, sieve pore (callus filled); *PrW*, primary wall; *ScW*, secondary wall; *Sh*, shredlike body; *V*, vacuole.) Fig. 1, cambial initial, terminal portion. Fig. 2, median portion. Fig. 3, young sieve tube, terminal portion. Fig. 4, median portion showing crumpled nucleus. Fig. 5, callus initiation in pit. Fig. 6, callus formation in pits. Fig. 7, callus rods complete. Fig. 8, definitive callus and shredded bodies in senile sieve tube.

streaming, thick, viscous cytoplasm; but during the late spring and summer the size of the oil globules is reduced, and the layer of cytoplasm becomes thinner and streams more rapidly. Plastids (fig. 2*Pl*) and mitochondria (fig. 2*M*) occur in the cytoplasm but are often difficult to distinguish, owing to fluctuations in the opacity of the cytoplasm. The nucleus (fig. 2*N*) is a long, spindle-shaped body lying against one wall near the middle of the cell. It is rather densely granular and contains several nucleoli (fig. 2*Nc*) (1).

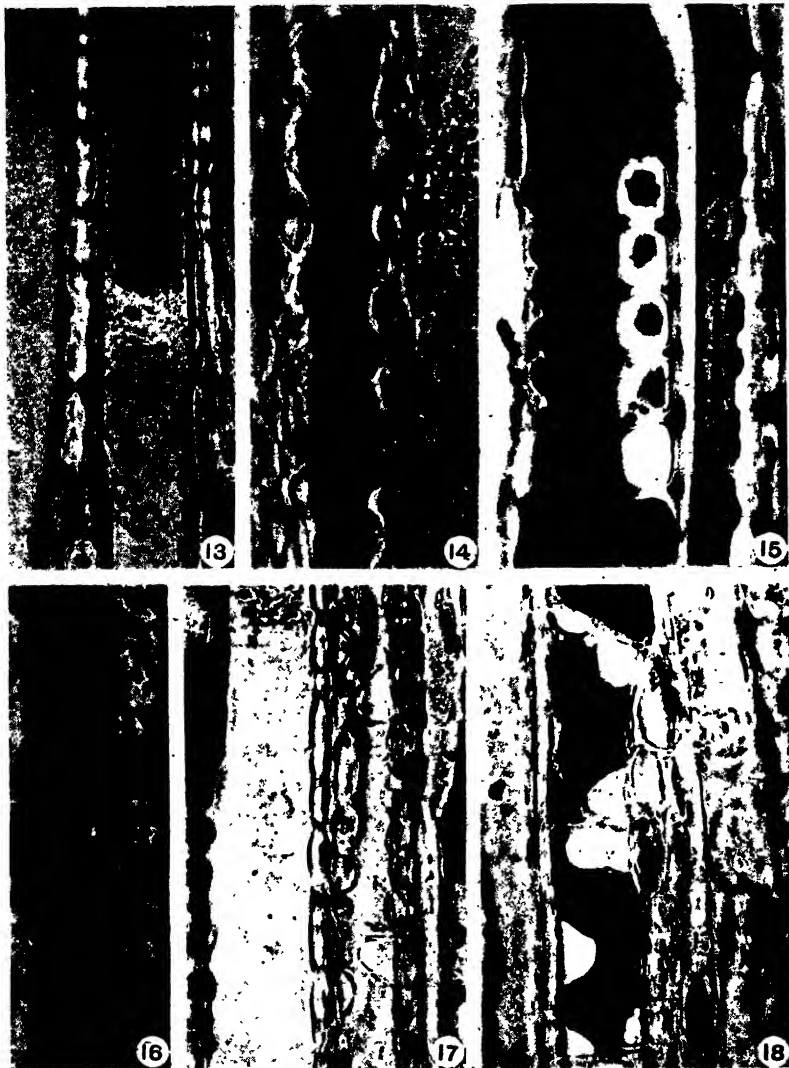
In Massachusetts, one of the first indications of growth in the spring is the enlargement of sieve tubes which failed to complete their differentiation during the preceding fall. Cambial cell division is relatively sluggish during March but is accelerated during April and May, resulting in the rapid formation of new xylem and phloem. Growth continues through the summer and fall at a diminishing rate, and sieve tubes initiated in late autumn may fail to complete differentiation until the following spring. Although young developing sieve tubes may at times be found in late November, the process of differentiation does not appear to be continuous throughout the winter. Given milder temperatures, sieve tube differentiation might proceed throughout the winter. This may account for the discrepancy between the writers' observations and those of STRASBURGER (21), who claimed that there are more differentiating stages in winter than during the growing season.

Indications of annual rings in the phloem of white pine (fig. 9) and of other Abietineae are an aid in studying the seasonal sieve tube increment. Sieve tubes which differentiate during the cooler, moister part of the year have slightly thinner walls and larger lumina than those found later, and the most conspicuous zone of parenchyma cells is formed in late spring in white pine (figs. 9, 10).

The young sieve tubes may be distinguished readily from young tracheids in tangential section by the following characteristics. The primary walls of the young sieve tubes are relatively thick, whereas those of tracheids are thinner. The plastids of the tracheids contain spindle-shaped bodies which stain blue-green in crystal violet and are distributed more or less evenly in the cytoplasm. The sieve tube plastids are located largely in the sieve areas (figs. 13, 14) and around the nucleus (fig. 13). They contain one to several granules at first,



FIGS. 9-12.—White pine. Fig. 9, transverse section of young phloem; $\times 200$. Fig. 10, longitudinal tangential section of mature phloem showing tanniniferous parenchyma, starch-bearing parenchyma, rays, and crystals; $\times 250$. Fig. 11, ray in longitudinal radial section; xylem on left, phloem on right; $\times 250$. Fig. 12, young sieve tube in bulging stage (right) and mature sieve tube (left); protoplasm with plastids at top of young element; living tissue with neutral red staining; $\times 500$.



FIGS. 13-18.—White pine. Fig. 13, young sieve tube in bulging stage showing protoplasmic bridge separating two vacuoles; living tissue with neutral red staining; $\times 500$. Fig. 14, end of young sieve tube in bulging stage showing neutral red stained vacuole, plastids in cytoplasm of pit areas, and starch grains in neighboring mature tube on right; $\times 1000$. Fig. 15, sieve tubes in material killed in IKI and stained with anilin blue; young sieve tube in bulging stage showing accumulation of cytoplasm in pit areas on left of ray, and more mature sieve tube with fibroid cytoplasm on right; $\times 500$. Fig. 16, tanniniferous phloem parenchyma cells in bulging stage; fixed material; $\times 500$. Fig. 17, plasmolyzed young sieve tube; $\times 500$. Fig. 18, plasmolyzed phloem parenchyma with sieve tubes unaffected; $\times 500$.

and later a single shell-shaped body which stains dark purple-blue in crystal violet. The younger sieve tube plastids are often covered with oil globules.

DETAILS OF SIEVE TUBE DIFFERENTIATION.—The early stages of sieve tube differentiation merge into one another but can be described in two steps. The wall and pit areas at first show little change except for a slight thinning down of the radial walls (fig. 19), especially during active growth in the spring.

In the spring, summer, and fall, the innermost sieve tubes (figs. 3, 4) have a layer of rapidly streaming, non-viscous protoplasm containing a large vacuole which stains orange in neutral red (fig. 14); whereas the vacuoles of the cambial initials stain magenta red. Imbedded in the cytoplasm, usually around the nucleus and in the pit areas, are the plastids (figs. 3, 4, 6, 13, 14). The usually roundish (fig. 13) or somewhat folded nucleus (figs. 4, 38) in such cells is more transparent than in the cambial initials, and has one to several nucleoli (see also 1, fig. 37). As differentiation proceeds, the nucleus swells slightly and becomes less elongate; the number of nucleoli is reduced. The oil globules become smaller and the cytoplasm streams more rapidly. Numerous leucoplasts may be observed at times in sieve tubes in this stage of development. These are plastic amoeboid bodies which change shape continually in the rapidly streaming protoplasm. They are found in cells which lack plastids with granules or have only a few such plastids.

TURGOR EXPANSION.—In the next stage (figs. 3, 4) cell enlargement occurs. The cytoplasm is in more active cyclosis, and there is evidence of internal pressures in many cells. The membrane of the pit areas often bulges outward (figs. 3, 12, 13, 14, 15), the pit enlarges, and its membrane becomes still thinner, owing to stretching. When two pits are close together, the wall between them often becomes rather thick at this stage, even before there is any evidence of secondary thickening (fig. 23). Slight thickening of the wall, due to apposition of secondary layers, may also be observed at times (figs. 3, 24, 26).

At this stage in sieve tube development, the parietal layer of cytoplasm becomes progressively thinner (figs. 12, 13). It streams around the cell in a steep spiral until it reaches the tip, where it



FIGS. 19-22.—White pine. Fig. 19, young sieve tubes and ray cells showing primary walls. Fig. 20, slightly older stage; tube on right shows some bulging; cytoplasm assuming parietal position and becoming fibroid and heavily staining; pitting to albuminous cells prominent. Fig. 21, slightly older stage of sieve tubes; highly fibroid cytoplasm torn in killing process, starch grains released into lumina. Diagonal wall between sister cells of recently divided initial shown in its primary condition. Fig. 22, definitive callus in senile phloem; all $\times 350$.

mills around and returns down the opposite side of the cell without apparently passing into the pit areas, which show only a slight Brownian movement of their contents.

The large numbers of plastids are mostly clustered around the nucleus (figs. 4, 12, 13) or in the pit areas (figs. 12, 14). At first each plastid contains several dark granules, and later the typical shell-shaped body which gives a pale purple or red color in iodine-potassium-iodide. The reaction has already been referred to by STRASBURGER (21), HILL (16), and others. The "starch grains" in mature sieve tubes of many plants give this same reddish color in iodine-potassium-iodide (4), and since in polarized light they are optically birefringent, it seems safe to assume that they are composed of starch or some related carbohydrate.

The nucleus is usually roundish, nearly filling the lumen at the middle of the cell (fig. 13). Unlike the cambial nucleus, it usually divides the vacuole in two. There are one to several nucleoli, and the nucleus often appears to be crumpled (see also 1, fig. 37).

Up to this stage of differentiation, the sieve tubes are capable of being plasmolyzed (fig. 17). The vacuoles take up neutral red from dilute buffer solutions relatively rapidly (figs. 12, 13, 14), and the cytoplasm continues to stream. These vacuoles are of the type B of BAILEY and ZIRKLE (3).

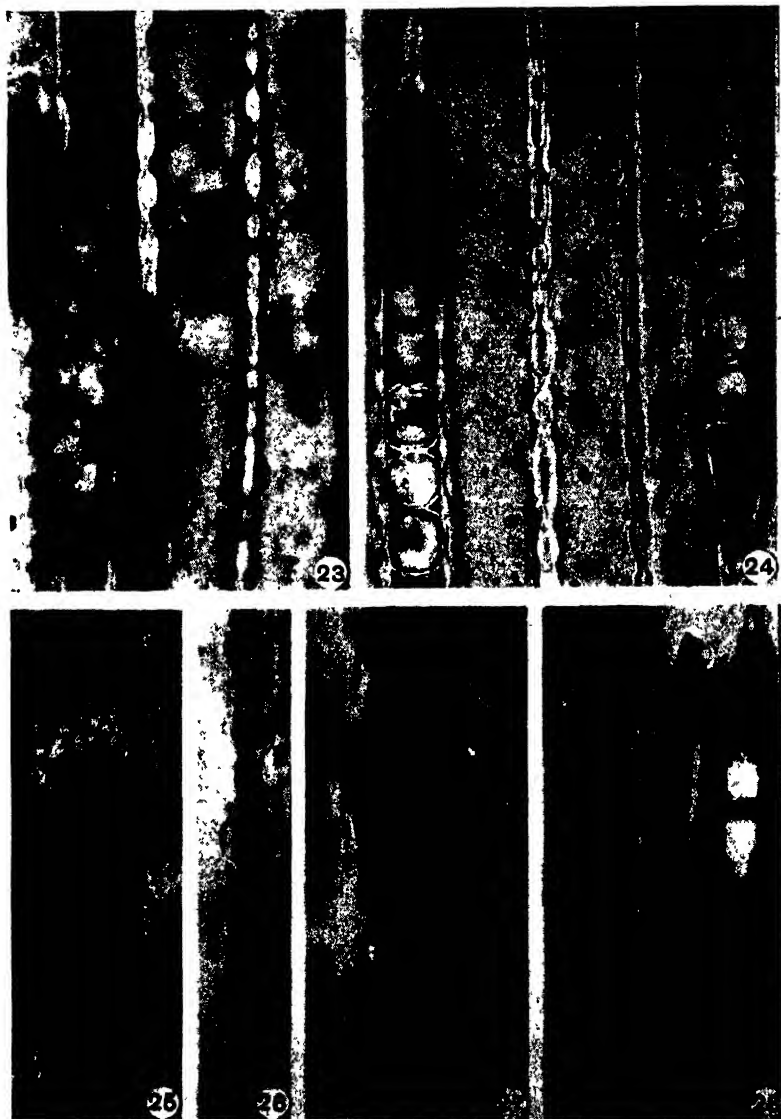
The bulging type of enlargement just described also occurs in young tracheids and parenchyma cells (fig. 16), and indicates that growth is a concomitant of high turgor pressure.

SIEVE TUBE MATURATION.—With the attainment of mature dimensions, the sieve tubes undergo changes of profound importance from both morphological and physiological standpoints. The nucleus of each cell, commonly located midway between the ends in a protoplasmic bridge dividing the lumen into two large vacuoles (fig. 13), expands and becomes less dense, often rumples (fig. 4), and finally fragments (fig. 23) and disintegrates, leaving no visible remnants. Attending this nuclear disintegration, the cytoplasm assumes a truly parietal position, as illustrated in the cell on the right of figure 23, passes through a stage when it becomes reticulate (fig. 25), and then fibroid (figs. 27, 28, 29). Finally assuming a truly parietal position, it loses its affinity for plasma stains and becomes

so thin and transparent that it is detected only with difficulty. Meanwhile the cell loses its ability to accumulate vital stains and can no longer be plasmolyzed. Figure 17 shows, at the right of center, a plasmolyzed young sieve tube, and at the left a mature element that has failed to respond to the hypertonic sucrose solution. Figure 18 shows mature phloem with plasmolyzed parenchyma cells of the A-vacuole type and two mature sieve tubes that are unaffected. In mature sieve tubes no vacuole (in the usual sense of the word) may be found, the interface between cytoplasm and the sap within the lumen being almost indistinguishable. The plastids break down, and their granules may be found later in the vacuolar sap, violently agitated by Brownian movement (fig. 6).

During this same ontogenetic period, the thin pit-closing membranes of the young sieve tube wall (fig. 24) thicken (figs. 26, 27), and the protoplasmic connections, demonstrable up to this time only by special methods involving severe swelling and dense staining (5), become visible by the water blue, iodine method (figs. 27, 28, 29). Careful study, using critical illumination and the best of optical equipment (Zeiss pancratic condenser; apochromatic 60 \times , 1.4 NA objective; and 15 \times Mobimi paired oculars), indicates that this increase in visibility is not due to any great increase in diameter of the threads but to the fact that they acquire a strong affinity for dyes and stain intensely. Furthermore, there is no evidence that they are "bored" out or transformed to "slime," as suggested by HILL (16). They later lose much of this affinity for dyes, just as does the parietal layer, and they are lost, together with the parietal layer, by dissolution when the element ceases to function. If the term slime is to be limited to the densely staining, finely particulate or colloidal material in the sieve tube lumen that results from disintegration of the nucleus, and in some species of slime bodies, then this material can be shown to be inclosed within the parietal protoplasm and to be distinct and separable from it. The slime is transitory in nature and soon disappears from the sieve tube, while the parietal cytoplasm, and the connecting threads of the sieve areas that are continuous with it, remain and can be demonstrated until lost at death of the cell.

Two other notable changes occur during this critical period in



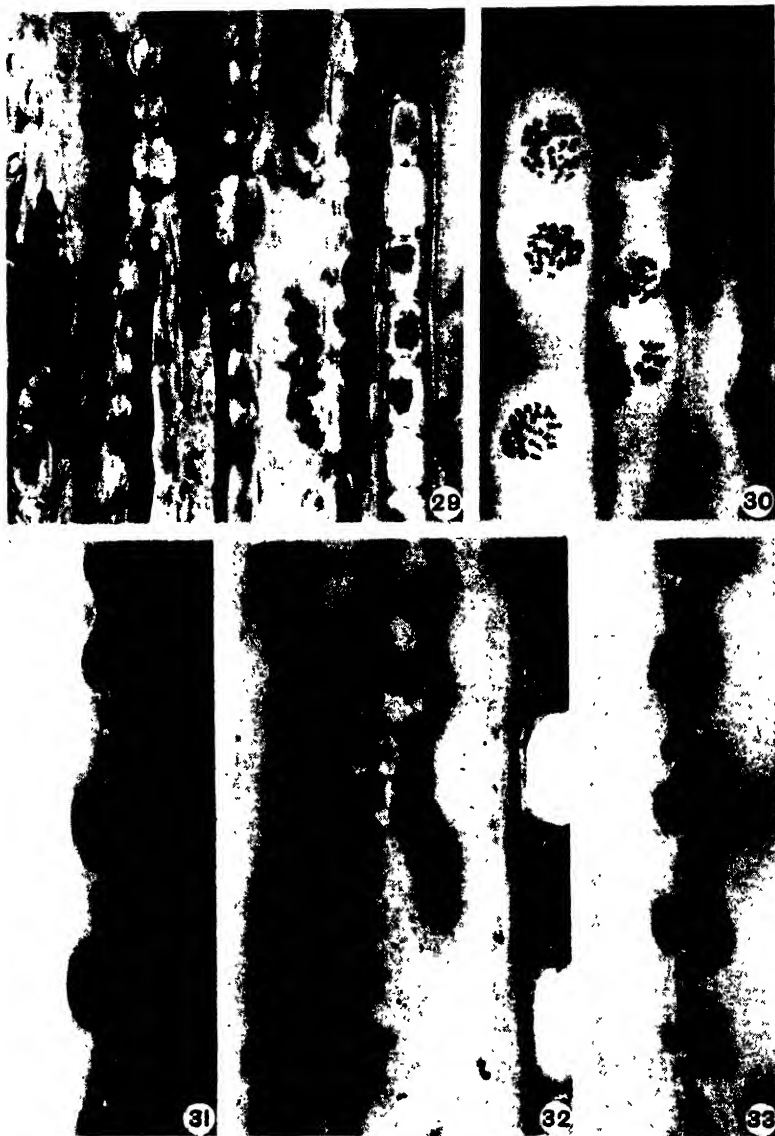
FIGS. 23-28.—White pine. Fig. 23, nuclear disintegration in young sieve tube; $\times 500$. Fig. 24, first stages of secondary wall formation with plastids still present in cytoplasm; $\times 500$. Fig. 25, sieve tube cytoplasm entering fibroid condition and increasing in staining affinity; $\times 1000$. Fig. 26, common wall between two young sieve tubes; pit-closing membranes thickening and callus formation just starting; protoplasmic connections visible under microscope; $\times 875$. Fig. 27, callus formation comparable with fig. 6. Note stringy condition of cytoplasm; $\times 1000$. Fig. 28, threads in wall more clearly visible, although callus rods not completed; $\times 875$.

sieve tube ontogeny. Attending the denaturing of the cytoplasm that occurs when the nucleus is lost, each individual thread (or small group of threads) traversing the sieve tube wall is surrounded by a callus cylinder or "rod." This material appears in small areas surrounding the protoplasmic threads of the sieve pits (figs. 5, 26), and increases inward (figs. 6, 27) until the cylinders converge at the middle lamella (figs. 7, 31, 32, 33). Figure 30 shows the ends of these rods as seen in radial longitudinal section. Figures 5, 6, and 7 illustrate successive stages in callus formation, from the initiation of rods to their completion.

The other important change taking place during sieve tube maturation is the development of secondary walls. This phenomenon is apparently restricted, among the species studied, to the Abietineae—the sieve tubes of Araucarineae, Taxodineae, Cupressineae, and Taxineae showing no evidence of a secondary wall. Figures 5, 6, 7, and 8 diagram this secondary thickening. Figure 23 shows the primary walls of young sieve tubes, figure 24 shows the beginnings of secondary thickening, and figures 26 and 28 show further development. In figures 27, 31, and 33 the walls were swelled somewhat with 5 per cent H_2SO_4 and stained with IKI. With this treatment they turned a light lavender and photographed darker. Note also the swelling and dense staining of the intercellular substance brought about by iodine and anilin blue in figures 27, 31, and 33. The median nodules described by HILL and others were black after this treatment.

When viewed in radial longitudinal section, the pits resulting from secondary wall formation are elliptical and oriented at about 90° in the walls of adjacent cells. This results in the peculiar skewed formation of the secondary thickenings in figure 31, which was cut somewhat off center as compared with figure 33 that more nearly bisected the pits. The Abietineae form the only group in which secondary wall thickening has been shown to occur in sieve tubes.

Radial and tangential sections of white pine phloem, when viewed in polarized light between crossed Nicols, showed a striated structure of the secondary walls from the latest streaming stages on. There was no evidence of the loss of this layer in the older sieve tubes. Previous to cell enlargement, the writers found no evidence



FIGS. 29-33.—White pine. Fig. 29, phloem showing extreme fibroid condition of cytoplasm and its intimate relation with sieve pits; $\times 500$. Fig. 30, sieve pits in longitudinal radial view showing ends of callus cylinders; $\times 1000$. Fig. 31, sieve pits in wall swelled with 5% H_2SO_4 , showing connecting threads and secondary wall structure; $\times 1000$. Fig. 32, similar view but with less swelling; callus rods complete as in fig. 7; $\times 1000$. Fig. 33, view like fig. 31, showing median nodules, callus cylinders, and swelled secondary wall thickening; $\times 1000$.

of spiral structure in the cambial or young sieve tube walls of this species. After swelling in 30 per cent sodium hydroxide and staining in iron-alum haematoxylin, spiral striations corresponding to those seen in polarized light were distinct in the radial and tangential walls of the older sieve tubes.

The following test for spiral arrangement was positive for the secondary layers of the sieve tube walls of white pine. Starting with transverse sections of the phloem, a series of increasingly oblique sections was made until the longitudinal plane was reached. It was found that as the plane of section became more oblique, the secondary layers of the wall on one side of the sieve tube became brighter while those on the other side became darker in polarized light, until a certain plane of obliquity was reached. Presumably, when the plane of section was parallel to the longitudinal axis of the micelles on one side of the cell, it was cut transversely to those on the opposite wall. Under these conditions the wall on the former side showed the maximum brilliance, while the latter was dark in polarized light.

During the mature functioning stage, the sieve tubes contain many small spherical, discoid, or shell-shaped starch grains having the same size and appearance as those found earlier in the plastids. The sap, with its included starch grains, can be forced from end to end of the cell by slight pressure on the cover slip, and when a sieve tube element is cut and slightly pressed, the sap and starch grains flow out rapidly.

As early as late August, transitional stages to the winter condition of the mature sieve tubes may be found in white pine. These transition types may show some starch grains but also contain a number of small shredlike bodies (fig. 8). The thicker portions of these shreds stain red in Sudan III and IV. By the time that near or below freezing weather has come, a large proportion of the mature sieve tubes has passed into this shred-containing condition. The shreds are sometimes as much as $3\ \mu$ wide and $10\ \mu$ long, and are quite refractive. They are very numerous in portions of the sieve tube lumen, where they may be found collected into masses, oscillating at various rates, the smaller ones relatively rapidly.

The smaller granule type of plastid body of the sieve tube in its streaming stage and the shreds of the winter condition are preserved

by osmic acid. On the other hand, the shell-shaped starch grains of older plastids and of the mature sieve tubes are not found in sections which have been fixed in Flemming's solution and imbedded, sectioned, and stained by the usual techniques. Evidently the starch is hydrolyzed by these methods.

PARENCHYMA OF PHLOEM.- Concurrent with sieve tube differentiation, parenchyma of two general types develops in white pine phloem. These are ray cells (fig. 11) and phloem parenchyma formed from fusiform initials by septation (figs. 9, 10).

Ray initials give rise to two distinct types of xylem cells, ray parenchyma and ray tracheids, the latter lying horizontally along the margins of the rays. In the cambium the young ray parenchyma stain densely with anilin blue and appear dark in both radial (fig. 11) and tangential view. Soon, however, the erect cells of the phloem rays exhibit conspicuous pitting (figs. 15, 19, 20, 29) toward the adjacent sieve tubes. These are the "albuminous" cells of STRASBURGER (21); (see also 15). They have been considered as analogous to the companion cells of angiosperms, because of their close association through pits, their dense protoplasmic content, and the fact that they die and are crushed along with the associated sieve tube elements. How much farther the analogy may be carried is doubtful, since these cells do not arise from a common fusiform initial with the sieve tube but from separate ray initials.

The remaining cells of the ray, most of which are horizontally elongated (fig. 11), soon vacuolate highly, become filled with starch or oil globules, and assume a relatively translucent appearance in tangential sections (figs. 10, 15, 19, 20, 21, 22, 24, 29). These cells persist after the death of the marginal cells and sieve tubes, and form, in the old cottony bark, a laterally arranged system of living cells providing conduction of nutrients to the phloem parenchyma and cork cambium. They increase in size, particularly by periclinal expansion, and remain alive until cut off by the phellogen. All the living ray cells have B vacuoles during the winter, and the persistent parenchyma cells just described contain numerous fat globules.

Phloem parenchyma formed by septation of fusiform initials is laid down relatively abundantly during the spring, forming annual

rings in the phloem by their radial alternation with bands of sieve tubes. Certain of these parenchyma cells may soon become filled with crystals and resin and die (fig. 10). Others vacuolate highly and store starch or oil in varying amounts (fig. 10, the clearer cells in the center). In many ways these cells compare with the horizontally elongated ray cells.

The great majority of the phloem parenchyma, however, rapidly develop large A vacuoles, become laden with tannin-like substances, and stain densely in fixed sections (figs. 9, 10, 16, 18). These cells form the layers of living tissue that persist in the older bark. Connected by living ray cells, these layers expand and crush the sieve tubes, gradually occupying the majority of the available space in the soft bark. They contain starch in addition to tannins during the autumn, and oil globules in the colder winter months. By renewed meristematic activity, certain of these cells give rise to successive cork cambia which produce the phellem of the outer bark.

DEATH OF SIEVE TUBE.—It is characteristic of the sieve tubes of all plants that, after a relatively brief functioning period—consisting of from a few days in the case of protophloem sieve tubes to a single season in most woody plants—the elements collapse, and death occurs. This is a necessary result of the nuclear disintegration and loss of semipermeability that initiate the functioning period of these elements. With continued reduction in the activity of the sieve tube cytoplasm, callus, which appears at the inception of this change, continues to develop. The callus rods of the sieve areas lengthen and enlarge, finally converging and forming the masses termed definitive callus (fig. 8). These masses are seen lining the sieve tube walls in figure 22, and in later stages may completely fill the lumina. Although this callus development may stretch the protoplasmic threads to many times their original length, there is no evidence that it “closes” the sieve “pores” in a physiological sense.

Definitive callus is simply a proliferation of the normal callus rods of the sieve areas, and occurs to some extent in all sieve tubes at the end of their functioning period. Callus may attain its ultimate development by the end of autumn in secondary phloem, and even earlier in primary phloem.

In white pine it persists in various stages of dissolution through-

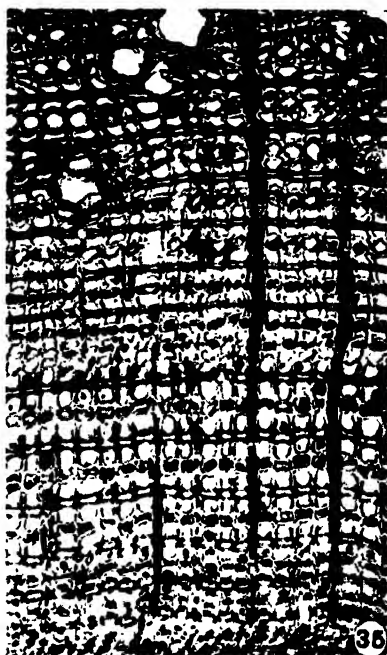
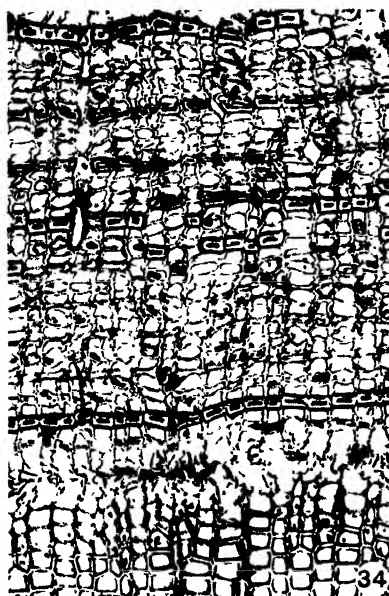
out the second season, and traces may be found in even older sieve tubes. The winter of the first year marks the end of the functional period of most of the sieve tubes however, for as the callus starts to disappear the parietal cytoplasmic layer dissolves. Air enters through the open lattices of the sieve areas of the dead sieve tubes, giving them a white glistening appearance. The walls become somewhat thinner as they dry. With drying, striations become more conspicuous in the secondary walls, emphasizing the characteristic helical structure.

Starch-filled phloem parenchyma cells persist in the white cottony old phloem tissue. This remains alive for many years, to form the thick bark of the mature white pine tree.

SIEVE TUBES OF OTHER CONIFERS

Figure 34 pictures the phloem of redwood, *Sequoia sempervirens*, and figure 35 that of incense cedar, *Libocedrus decurrens*. In contrast to white pine, these species have phloem fibers and show a stratified bark. The standard pattern of radial alternation of phloem elements in these species is fiber: sieve tube: phloem parenchyma: sieve tube: fiber, etc.; but examination of the figures shows some variation in pattern and considerable difference in the degree of wall thickening in different fiber layers. It is interesting to note that in the Cupressineae, Taxodineae, and Taxineae, secondary thickening is apparently restricted to the phloem fibers; whereas in Abietineae, where fibers are lacking, it is limited to sieve tube walls.

Figure 38 shows the very young phloem of redwood, a rumpled nucleus being present in the sieve tube slightly to the left of the ray in this picture. The walls are quite thin. Figures 39, 40, and 41 show the three tissues of functional phloem: sieve tubes (fig. 39), phloem fibers with their thick secondary walls (fig. 40), and phloem parenchyma (fig. 41). Again, phloem parenchyma cells are of two types, as are those of the rays. Albuminous cells are relatively fewer in number than in white pine. These cells are heavily pitted where in contact with sieve tubes. When the latter have definitive callus on their sieve areas, it occurs on the sieve tube side but is lacking on the ray cell side of their common pit pairs.



FIGS. 34-37.—Fig. 34, redwood phloem in transverse section with xylem at bottom and cambium somewhat crushed; $\times 200$. Fig. 35, incense cedar phloem in transverse section with cambium at bottom and resin cysts near top; $\times 200$. Fig. 36, definitive callus in senile phloem of fir; $\times 350$. Fig. 37, slightly calloused sieve pits of same in longitudinal radial section; $\times 1000$.



FIGS. 38-42.—Redwood. Fig. 38, very young phloem, sieve tube with crumpled nucleus. Fig. 39, mature phloem showing thickened primary walls with sieve pits. Fig. 40, phloem fibers in mature phloem showing secondary wall thickening. Fig. 41, phloem parenchyma with starch-bearing cells on extreme right and tanniniferous cells in remainder of section. Fig. 42, definitive callus in senile sieve tubes; all $\times 350$.

Figures 42 and 44 show definitive callus in the sieve tubes of *Sequoia sempervirens*, and figures 36 and 37 show it in phloem cells of the root of *Abies concolor*,—figure 36 in longitudinal tangential view and figure 37 in longitudinal radial view.

Threads in the sieve areas of *Sequoia sempervirens* are shown in figures 43, 45, and 46. They differ in this species from the other species studied in being extremely fine and forming a somewhat anastomosing network within the group involved in a single callus rod. This is particularly prominent in the connections of sieve tubes the protoplasm of which is in the fibroid, elastic condition just after disintegration of the nucleus. Figures 45 and 46 show the stringy nature of the parietal layer and internal strands during this phase of sieve tube ontogeny.

Figure 44 shows an early stage in definitive callus formation in *Sequoia sempervirens*; figure 47 a similar stage in *Abies concolor*.

The principal difference between the contents of the sieve tubes of the several conifers studied was found to be in the size and amount of the particles occurring in the elements during the winter. In *Taxus*, *Taxodium*, *Thuja*, and *Juniperus*, the large shreds noted in the Abietineae during the winter months were lacking, and fewer smaller bodies were observed in the sieve tubes. In some cases the sieve tubes were almost empty of visible contents except for large callus caps over the sieve areas. Callus formation was found to be essentially similar in all species studied. Sieve tube crushing was delayed in the Abietineae, as compared with the other species studied, probably owing to the secondary wall thickening. These elements persist, often for many years, in a semicollapsed condition, filled with air and devoid of protoplasm.

Microchemical tests on the walls of the various species studied show that the intercellular substance contains a high percentage of polyuronides; the primary walls are largely cellulose but contain some polyuronide; and the secondary walls of the sieve tubes of the Abietineae appear to be composed of relatively pure cellulose. While lignified fibers occur in many species, no lignin test could be obtained from any sieve tube walls.

In contrast to white pine, the writers were unable to find evidence of spiral structure in the sieve tube walls of *Juniperus virginiana*,



FIGS. 43-47.—Fig. 43, sieve tube walls of mature redwood phloem; $\times 1000$. Fig. 44, early stage in definitive callus formation on sieve pits of redwood; $\times 1000$. Fig. 45, cytoplasmic connections in sieve pits of redwood sieve tubes in swelled section; $\times 1400$. Fig. 46, same showing details of anastomosing branched sieve connections; $\times 1400$. Fig. 47, early stage in definitive callus formation in tr; $\times 1000$.

either under polarized light or by the use of the swelling and staining agents.

When transverse sections of *Sequoia sempervirens* were treated in 30 per cent sodium hydroxide and subsequently stained in chloro-zinc iodide, an inner wall layer was differentiated by its darker color, but it could not be proved to be secondary.

Discussion

Early literature on the phloem of conifers has been noted by HILL (16), and recent reviews (19, 12, 18, 9) cover the various aspects of phloem function. Most of the physiological interpretation of phloem structure relates to angiosperms (12, 18, 9). A brief review of the phloem anatomy of conifers might clarify some of the essential features of the mechanism that has been provided in these plants.

In contrast to the more specialized dicotyledons, the sieve tubes of conifers are very long and narrow. Cross walls of the type termed "sieve plates" are wanting, the sieve elements uniting longitudinally by diagonal common walls. Plasmodesmata between adjacent sieve elements are numerous but extremely tenuous. There are no companion cells, in the sense in which they occur in angiosperms.

On the other hand, sieve tubes in conifers resemble those in angiosperms in many ontogenetic features. They expand very rapidly, passing through a "bulging" stage; they lose their nuclei at maturity; their protoplasm goes through a similar series of changes, becoming fibroid, tough, elastic, and heavily staining; the mature elements often contain starch grains. Accompanying the denaturing process, the protoplast ceases streaming, loses its ability to accumulate solutes, and becomes completely permeable. In fact these protoplasmic changes, ending in complete permeability, seem to characterize sieve tubes in many plants (10), being the most constant feature of these elements that is known.

The enucleate permeable state of the mature functioning sieve tube in conifers predestines it to a short life, and, as in other vascular plants, these elements function for a season or less and then die, become air-filled and collapse or completely obliterate. This is shown graphically in figure 35. The sequence of cells in this section is sieve tube, parenchyma, sieve tube, fiber, etc. Starting at the cambium

along the lower edge, one may count twelve rows of differentiating cells to the third row of fibers, then twelve rows of mature elements, six of which are functioning sieve tubes. Above the sixth row of fibers, most of the sieve tubes are partially filled with definitive callus, denoting the end of their functioning life. Then there is a rapid change, the sieve tubes becoming smaller and flatter, the parenchyma cells larger and rounder, until between the twelfth and thirteenth fiber rows the latter cells are completely rounded and the sieve tubes badly crushed. This same course of ontogeny can be found in figure 34 and is characteristic of the phloem of all plants. It is not dependent upon cutting but may be found in stems killed *in toto*. It serves to emphasize the extremely narrow band of phloem tissue engaged in the longitudinal conduction of foods.

Rapid phloem exudation has recently been demonstrated in white pine (10). The technique necessary for the demonstration emphasizes again the nature of the mechanism and the limited band of tissue involved. In the experiments the hard bark and the soft white cottony phloem tissue is pared away, leaving exposed the thin, translucent, gelatinous layer of current season's phloem. When this is rapidly cut across, taking care not to go deep enough to open the xylem, several drops of a sweet sugary solution collect and flow from the wound. If any of the white cottony tissue is allowed to remain near the cut, the solution is rapidly absorbed into the old sieve tubes by capillarity, and the demonstration fails. Likewise if the xylem is cut, the solution is lost.

With the demonstration of the enucleate permeable condition of the sieve tubes of a wide variety of plants (10), there appears to be a rational basis upon which the structure of phloem in gymnosperms and angiosperms may be reconciled. Emphasizing the fundamental similarity in the mechanism of organic solute transport in all vascular plants, it points to a solution of this old problem by the demonstration of permeable conduits suitable to rapid longitudinal flow.

The tough fibroid nature of the parietal cytoplasmic layer in the enucleate sieve tube element has been described in cucurbits (6, 9), potato (7), and tobacco (8, 13). It is illustrated for white pine in figures 15, 25, 27, and 29, and for redwood in figures 45 and 46. In figure 15 the cytoplasm in the cell on the left of the ray was in a fluid

condition normal to living parenchyma cells. In the slightly more mature element on the right it was beginning to change, having shrunken and pulled away from the walls. In figures 25, 27, and 29, successive stages in the denaturing process are shown, the latter showing the heavy staining phase in which plasmodesmata are so prominent. Later, while remaining thin and fibroid, the parietal layer loses its affinity for stains and is demonstrated only by special methods. All attempts to find protoplasmic streaming in such protoplasm have failed, Brownian movement of the starch particles being the only motion observed.

CURTIS (11, 12), FISCHER (14), and others have objected that any method involving cutting the sieve tube system may fail to give a true picture of its contents. Therefore a branch of a five-year-old pine was shaved down, leaving a short thin continuous strip including the pith, wood, cambium, and bark. In such strips the inclusions of intact mature sieve tubes are in Brownian movement, and do not exhibit cyclosis.

Many phases of phloem structure still require investigation. The question of the sieve tube: companion cell relation is an open one. Although the origin of the erect ray cells in white pine is different from that of companion cells of angiosperms, the close association of these two cell types with enucleate sieve tubes is interesting. The common pitting and simultaneous death and crushing of marginal ray cells and sieve tubes indicate an essential interdependence between these elements. The protoplasm of the functioning sieve tube can scarcely be considered dead, and its activities may possibly be controlled by some adjacent nucleate cell. THOMPSON (22) was obviously in error in his description of companion cells in *Gnetum*. The elements he described as companion cells arise from cambial initials that give off xylem parenchyma on the inside, and not from division of the sieve tube mother cell after leaving the cambium. They do not have the thin common walls with sister sieve tube elements characteristic of companion cells. They are not crushed with the sieve tubes, but persist, and in some species differentiate into gelatinous fibers. They are undoubtedly parenchyma-like in nature throughout the period of sieve tube functioning.

Although it is often difficult to generalize from structure to func-

tion in plants, current theories on solute transport in the phloem differ so widely in the activity required of sieve tube protoplasm that studies of the type reported here should offer some concrete clues. It seems obvious that any hypothesis requiring rapid protoplasmic streaming, or a high activity state in the mature sieve tube protoplasm, is not supported by the cytological and anatomical data presented in this paper.

Summary

1. In Massachusetts, cambial division in white pine starts as early as February in some years, and is rapid during May. Phloem differentiation may lag until late summer or early fall, and daughter cells may remain in various stages of maturity through the winter.

2. Sieve tube differentiation starts with a localization of the nucleus in a protoplasmic bridge in the center of the cell. Rapid turgor expansion follows, bulging the pit areas and enlarging the cell. Protoplasmic streaming and vital stain accumulation indicate a high state of cell activity. As the sieve tube matures the nucleus disintegrates; the cytoplasm ceases streaming, becomes fibroid in consistency, develops an affinity for stains, and assumes a parietal position in the cell. The cell walls thicken, secondary walls are formed, and the connecting protoplasmic threads of the sieve areas stain heavily. Cessation of streaming, loss of the power to accumulate vital stains, and absence of plasmolysis in the presence of hypertonic solutions indicate a low activity state and a high permeability of mature sieve tube cytoplasm.

3. Denaturing of the protoplasm of the connecting threads is attended by callus formation in their immediate vicinity. Starch grains in the sieve tube plastids are released into the lumen and float free in the liquid contained there.

4. Senility of the sieve tube is attended by increase in the volume of callus on the sieve areas, a thinning down and loss of staining affinity by the cytoplasm, and a reduction in the numbers of starch grains.

5. Death of the sieve tube results in loss of the cytoplasm and callus, invasion of the lumen by air from the outer bark, and finally collapse of the elements.

6. White pine phloem contains no fibers; secondary wall thickening occurs in sieve tubes. Other genera of conifers have phloem fibers, and secondary wall thickening is confined to these elements, which are lignified.

7. Protoplasmic connections in the sieve areas of white pine are slender, solid, and fibroid in structure throughout the functioning period. Those of redwood are even more slender, consisting of anastomosing strands.

8. The picture of the functioning sieve tube of the conifers developed during these studies offers little support to theories of phloem transport requiring protoplasmic streaming or a high activity state in the mature sieve tube cytoplasm.

The investigations reported in this paper were initiated and conducted independently by the two writers. Since they dealt with the same plants from somewhat different points of view, it seemed advisable to combine the results in a single publication. Much of the cytological and histological work was carried out by Mrs. ABBE; the physiological investigations—particularly the plasmolysis and exudation experiments—were done by Dr. CRAFTS. The writers wish to express their appreciation to Professor I. W. BAILEY, of Harvard University, for assistance throughout the course of the work.

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✓ HISTOLOGICAL STUDY OF THE DEVELOPING FRUIT OF THE SOUR CHERRY¹

II. B. TUKEY AND J. ORAN YOUNG

(WITH EIGHT FIGURES)

Introduction

Earlier workers in the morphology of drupe fruits, as LAMPE (11), TSCHEIERSKE (19), FARMER (8), and WINTON (24), dealt primarily with the structure of the mature fruit, and recorded but few significant structural changes in the developing fruit. For these studies, various species of *Rubus* were largely used, as *R. ideaus*, *R. fruticosus*, *R. strigosus*, and *R. occidentalis*, although LAMPE included a brief comparative discussion of the sour cherry, *Prunus acida*.

Later workers have been more concerned with the external or gross changes of the developing fruits, and have dealt principally with species of *Prunus*, as CONNORS (5), BLAKE (2), LILLELAND (13), HARROLD (9), DORSEY and McMUNN (6), and TUKEY (21) with the peach (*Prunus persica* Stokes); LILLELAND (12, 14) with the apricot (*P. armeniaca* L.) and plum (*P. domestica* L.); and TUKEY (20, 22) with the sweet cherry (*P. avium* L.) and sour cherry (*P. cerasus* L.). These studies showed three characteristic growth periods in the developing fruit, designated (22) as: stage I, a period of rapid enlargement beginning about the time of full bloom; stage II, a mid-season period of retarded development varying in duration as correlated with the class of fruit and the season of fruit ripening; and stage III, a second period of rapid enlargement extending to fruit ripening (fig. 1).

Further study showed gross morphological changes of different parts of the fruit, such as the stony pericarp, nucellus and integuments, endosperm, and embryo. These were correlated with the three major growth stages of the fruit by TUKEY (20, 21, 22), LILLELAND (14), and HARROLD (9). LOTT (15), and ADDOMS, NIGHTINGALE, and BLAKE (1) showed chemical changes in the

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fleshy and stony pericarp, and LOTT (15) and TUKEY and LEE (23) in the seed and embryo as related to these stages.

The histological and structural changes have been less well investigated. The present study is an attempt to show these changes in the developing fruit of the sour cherry (*Prunus cerasus* L. var. Montmorency), from the closed bud (18 days before full bloom) to the ripe fruit. It includes a treatment of the principal tissues of the pericarp during each of the three growth stages and during the pre-bloom stage.

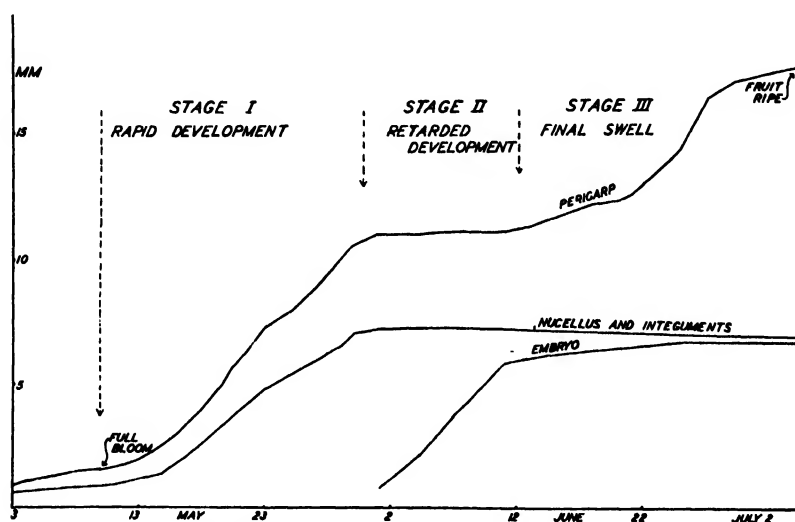


FIG. 1.—Growth stages in development of fruit of sour cherry from pre-bloom to fruit ripening. After TUKEY (22).

Material and methods

Montmorency, a mid-season variety, ripens 57 to 59 days after full bloom. In this variety stage I is of 20 to 22 days duration; stage II, 12 to 16 days; and stage III, 21 to 23 days (22).

MATERIAL.—Fresh material from the season of 1938 and material fixed in three different ways and during four different seasons were used, as follows:

1. Season of 1930, ripe fruit in 95 per cent alcohol.
2. Season of 1931, at 2 to 3 day intervals from April 20 to May 26, in Karpechenko's chromacetic and in formalin-acetic alcohol.

3. Season of 1932, daily from June 4 to June 26, in formalin-acetic alcohol.
4. Season of 1938, at 2 to 5 day intervals from May 24 to July 19, in formalin-acetic alcohol.

Although there was some variation in the number of days from full bloom to fruit ripening in the different years, it was possible to correlate the material. A growth curve was made for each season based on gross measurements of the fruit and examination of the development of the embryo and stony pericarp (fig. 1). The dates of full bloom and of fruit ripening being known, it was possible to locate definitely the beginning, middle, and end of each of the three stages of fruit development for the material from all four seasons, and to place one curve upon another. Samples from the various seasons were accordingly selected for study at these points and were found to fit together almost perfectly to form a four season record of development.

FIXATION, SECTIONING, AND STAINING.—Material used in the study of the pre-bloom stage and parts of stage I was killed and fixed in Karpechenko's chromacetic and run up into paraffin; then sectioned with a rotary microtome and stained with safranin, orange G, and crystal violet.

Larger specimens used in the study of stages I, II, and III to fruit ripening were either examined fresh or killed and fixed in a mixture of 100 cc. of 50 per cent alcohol, 23 cc. of glacial acetic acid, and 67 cc. of formalin, and held in this mixture until sectioned either freehand or on a sliding microtome. All freehand sections of this type were stained in safranin and light green. Most of them were mounted in balsam, but some were examined immediately in liquid mounts and found satisfactory for outline drawings.

When the stony pericarp had begun to harden (stage II), there was some difficulty in getting good sections which contained both stony and fleshy pericarp. Attempts at freezing and holding the fruit in various pin devices so as to exert no pressure on the fleshy pericarp were not superior to making numerous freehand sections and selecting the better ones for study. Sections made from fresh material and from that in formalin-acetic alcohol were equally satisfactory.

When the stony pericarp had become too hard to secure complete

sections through the entire pericarp, freehand sections for detailed study were made separately in three regions: the fleshy pericarp, portions of the adjacent stony and fleshy pericarps, and the stony pericarp.

For complete sections of the stony pericarp from ripe fruit, the pit was removed and ground successively on coarse sandpaper, on fine sandpaper, on a medium carborundum stone, and on a fine oil stone. When the section was 2 mm. thick, it was mounted on a glass slide with Duco household cement and ground further to the desired thickness. This cement hardened rapidly, held the mount firmly, and permitted staining without separation from the slide.

The epidermis was examined in surface view by stripping from the fruit. No stain was needed since the cells were sufficiently pigmented to show good contrast.

MEASUREMENTS AND DRAWINGS. All comparative outline drawings were made from cross sections through the center of the fruit perpendicular to the polar axis. Comparative measurements and drawings of number and size of cells were made from sectors at the cheek of the fruit, as this has been shown (22) to be the point at which development is most regular. The cheek diameter may be defined as a line passing through the center of the fruit perpendicular to both the longitudinal axis and the suture diameter.

Outline drawings where no cell details were desired were made with the use of a Bausch & Lomb triple purpose microprojector using a 16 mm. objective and a constant set-up. Detailed drawings were made with a camera lucida, always to the same scale, and with the same set-up of equipment to give identical enlargement.

Counts of numbers of cells and measurements of cell size were made with the use of both fixed and sliding ocular micrometers, and checked against camera lucida drawings. For cell size, an average was taken of several cells which seemed typical of the tissues under consideration. Mitotic figures were noted and recorded as evidence of cell division.

Investigation

GENERAL CONSIDERATIONS OF TISSUES INVOLVED

Three principal tissues compose the ovary wall of the cherry: inner and outer epidermis, stony pericarp, and fleshy pericarp. The

gross development of the fruit and of these tissues is shown in the accompanying series of outline drawings (fig. 2), beginning 18 days before full bloom and continuing to fruit ripening. The development at the beginning, middle, and end of each stage is shown.

The stony pericarp may be divided into an inner and an outer layer, and the fleshy pericarp into an innermost layer of small thin-walled parenchyma, a middle region of large thin-walled parenchyma, and an outer or hypodermal layer of collenchyma. The hypodermal layer and the epidermis together constitute the "skin" of the fruit. Not all of these tissues are sharply defined in the earlier stages of development, and they are outlined in the accompanying drawings only when they are to be easily seen at that stage.

In subsequent detailed drawings (figs. 3, 4, 5) of the cellular changes which these tissues undergo, the same relative positions on the page are retained for drawings of similar stages as those of gross development in figure 2, to facilitate comparison. In tables 1 and 2 the counts of cell number and measurements of cell size in the tissues involved are for corresponding stages of development. In the discussion which follows, the tissues are taken up in the following order: stony pericarp, fleshy pericarp, and outer epidermis.

STONY PERICARP

PRE-BLOOM.—Eighteen days before full bloom the innermost portion of the ovary wall, which becomes the stony pericarp at fruit ripening, is composed of the single row of cells of the inner epidermis and an additional three or four layers of cells of the pericarp (fig. 3A). The cells of the inner epidermis are block-shaped while the others are elongate in the direction of the central axis of the fruit.

The line of demarcation between that portion of the pericarp which is destined to become stony and that which is to remain fleshy is discernible even at the beginning of the period, the cells of the former being smaller, in general, than those of the latter. It is not possible at this date to separate a single bordering cell of the one from the other. The relatively smaller size of cells of the stony pericarp persists to maturity.

Soon after the beginning of this period the cells of the inner epidermis elongate tangentially, as seen in cross section, and just before full bloom they divide in a transverse direction (fig. 3B, C).

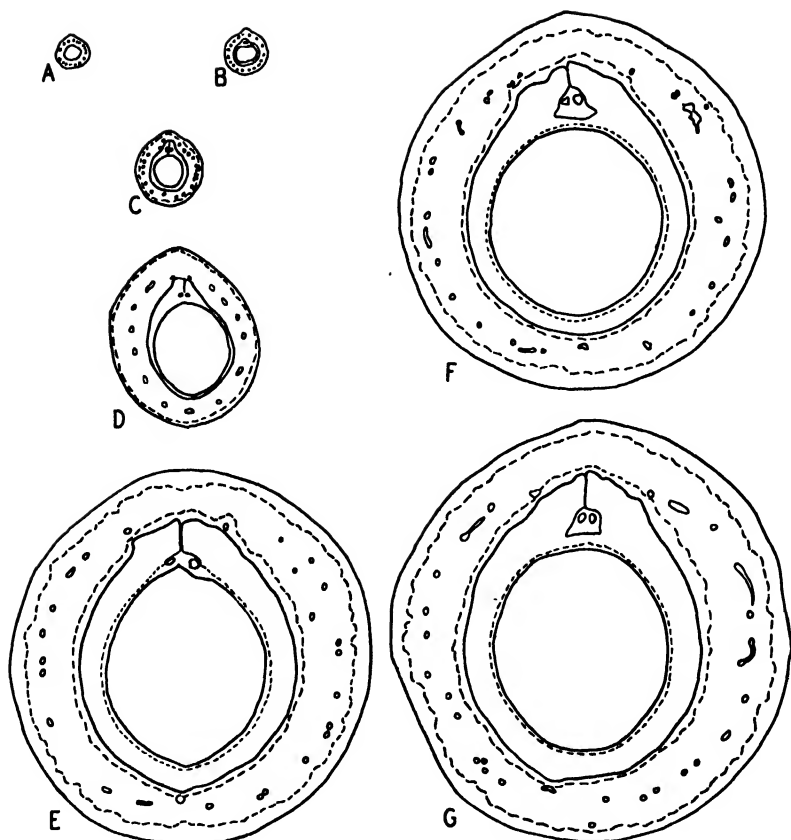
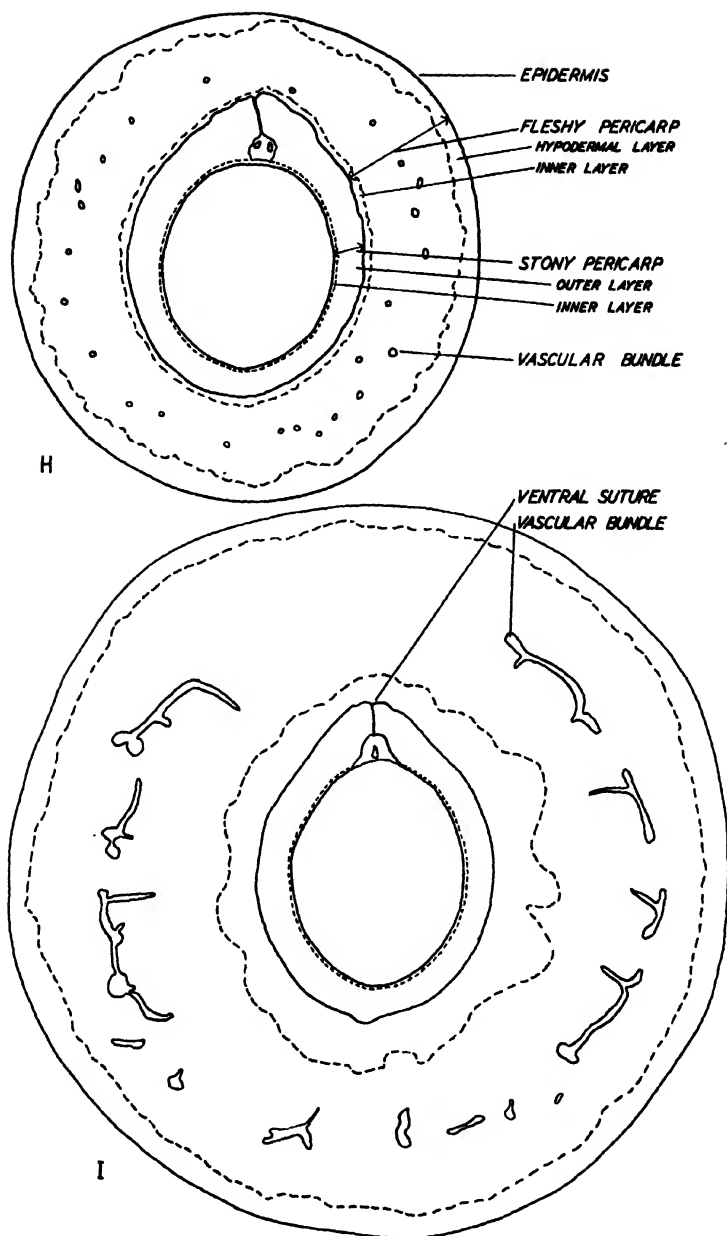


FIG. 2.—Development of cherry fruit as seen in transverse section, from 18 days before full bloom to fruit ripening. *A*: 18 days before full bloom; *B*: 6 days before full bloom; *C*: full bloom; *D*: 11 days after full bloom; *E*: 20 days after full bloom; *F*: 26 days after full bloom; *G*: 36 days after full bloom; *H*: 40 days after full bloom; *I*: fruit ripening, 57 days after full bloom.



(FIG. 2.—Continued)

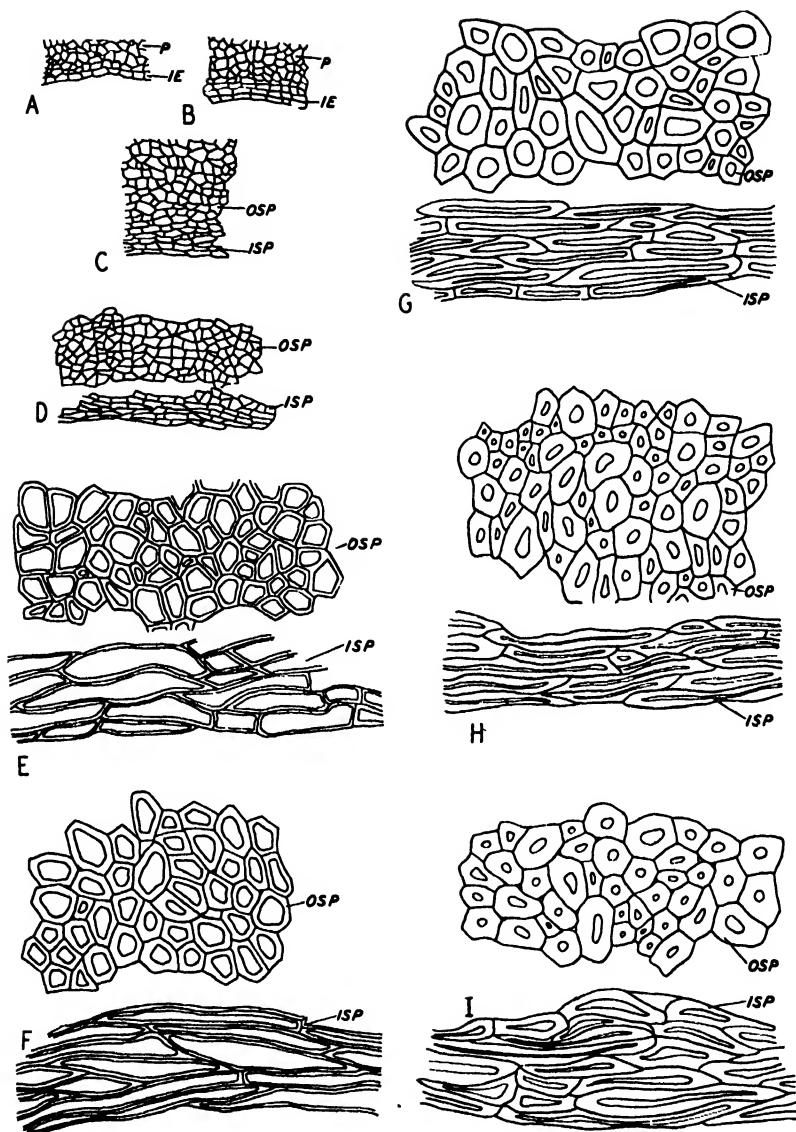


FIG. 3.—Development of stony pericarp (camera lucida drawings of transverse sections $\times 163$). *p*, pericarp; *ie*, inner epidermis; *osp*, outer stony pericarp; *isp*, inner stony pericarp. *A*: 18 days before full bloom; *B*: 6 days before full bloom; *C*: full bloom; *D*: 11 days after full bloom; *E*: 20 days after full bloom; *F*: 26 days after full bloom; *G*: 36 days after full bloom; *H*: 40 days after full bloom; *I*: fruit ripening, 57 days after full bloom.

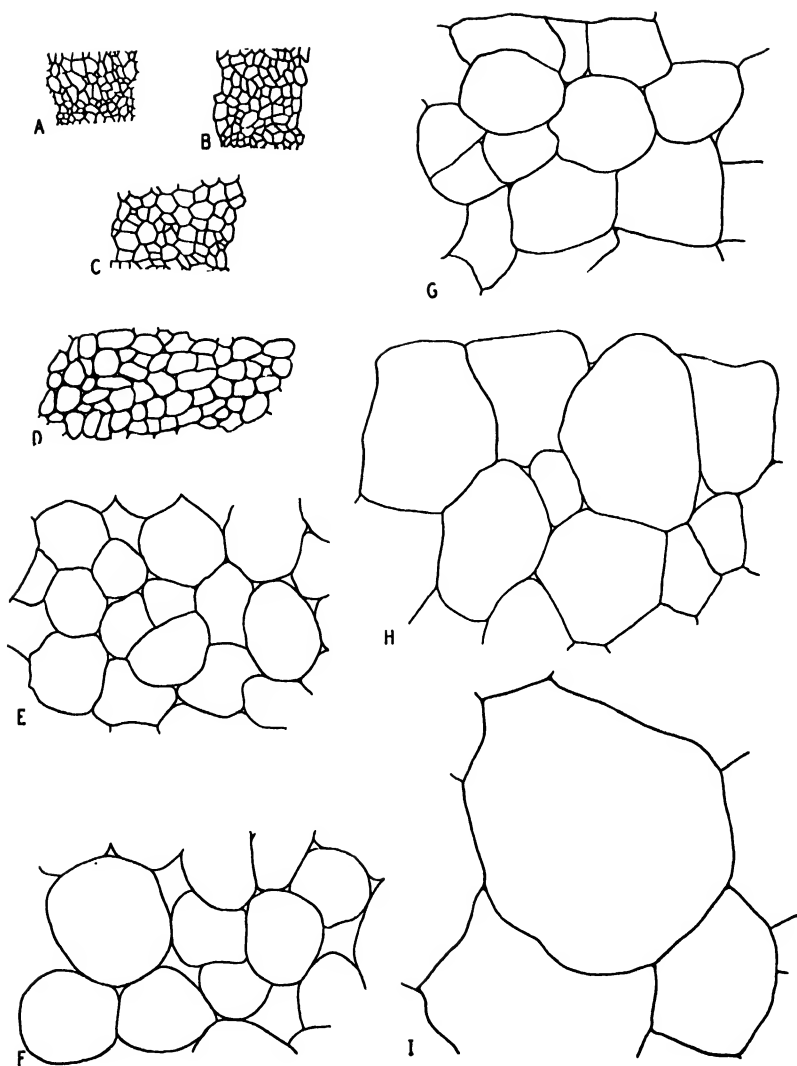


FIG. 4.—Development of fleshy pericarp, in region between hypodermal layer and ring of vascular bundles (camera lucida drawings of transverse sections $\times 163$). *A*: 18 days before full bloom; *B*: 6 days before full bloom; *C*: full bloom; *D*: 11 days after full bloom; *E*: 20 days after full bloom; *F*: 26 days after full bloom; *G*: 36 days after full bloom; *H*: 40 days after full bloom; *I*: fruit ripening, 57 days after full bloom.

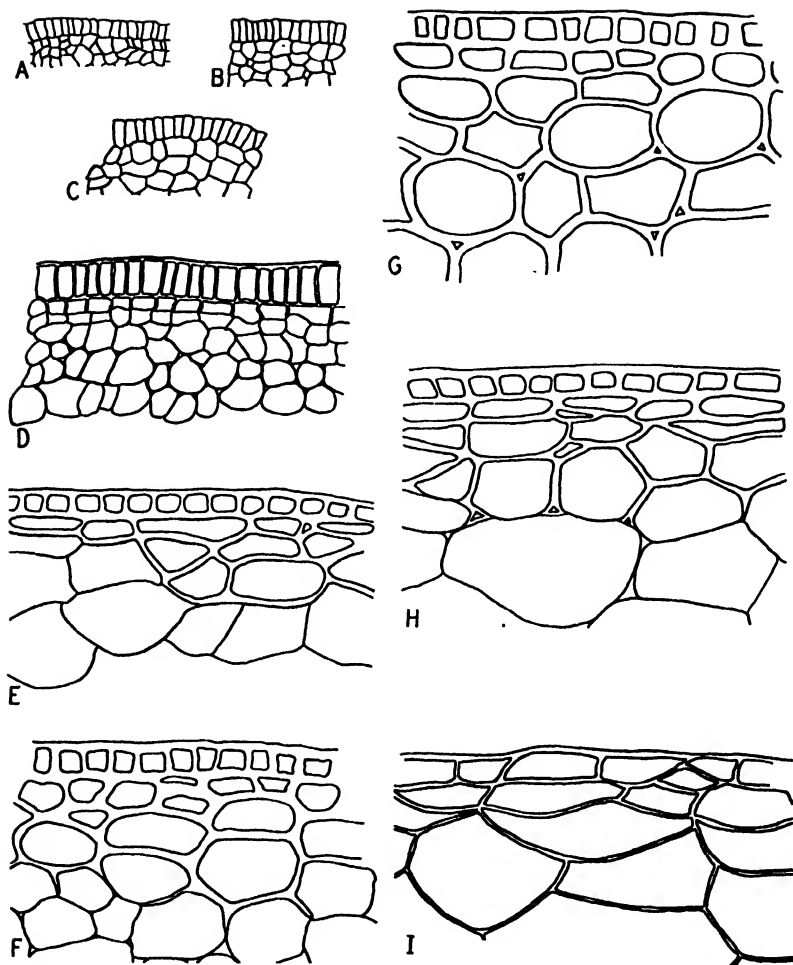


FIG. 5.—Development of epidermis and hypodermal layer (camera lucida drawings of transverse sections $\times 163$). *A*: 18 days before full bloom; *B*: 6 days before full bloom; *C*: full bloom; *D*: 11 days after full bloom; *E*: 20 days after full bloom; *F*: 26 days after full bloom; *G*: 36 days after full bloom; *H*: 40 days after full bloom; *I*: fruit ripening, 57 days after full bloom.

TABLE 1

CHANGES IN NUMBER OF LAYERS OF CELLS IN A RADIAL DIRECTION THROUGH
CHEEK OF CHERRY FRUIT FROM 18 DAYS BEFORE
BLOOM TO FRUIT RIPENING

STAGE OF DEVELOPMENT	DAYS BEFORE OR AFTER FULL BLOOM	FLESHY PERICARP				STONY PERICARP		
		EPIDER- MIS AND HYPO- DERMIS	LARGE- CELLED PAREN- CHYMA	SMALL- CELLED PAREN- CHYMA	TOTAL	OUTER	INNER	TOTAL
Pre-bloom.	-18	4-5	11	15-16	4-5	4-5
Pre-bloom.	- 6	4-5	11	15 16	7-8	7-8
Full bloom.	0	5	17	22	10	4	14
Middle stage I.	+11	5-6	19-21	24-27	12	6-7	18-19
End of stage I.	+20	5-6	18-19	3-4	26-29	17-18	6-7	23-25
Middle stage II.	+26	5-6	19	3-4	27-29	20	6-7	26-27
End of stage II.	+36	5-6	19	3-4	27-29	20	6-7	26-27
Middle stage III.	+40	5-6	19	3-4	27-29	20	6-7	26-27
End of stage III.	+57	5-6	19	3-4	27-29	20	6-7	26-27

TABLE 2

CHANGES IN SIZE OF CELLS IN A RADIAL DIRECTION THROUGH CHEEK OF
CHERRY FRUIT FROM 18 DAYS BEFORE FULL BLOOM TO FRUIT RIPENING

STAGE OF DEVELOPMENT	DAYS BEFORE OR AFTER FULL BLOOM	EPIDERMIS		FLESHY PERICARP		STONY PERICARP
		RADIAL DIAMETER (μ)	TANGEN- TIAL DIAMETER (μ)	PARENCHYMA		CROSS SECTION (μ)
				RADIAL DIAMETER (μ)	TANGEN- TIAL DIAMETER (μ)	
Pre-bloom.	-18	17	10	18	13	10
Pre-bloom.	- 6	18	10	18	16	10
Full bloom.	0	22	11	20	20	11
Middle stage I.	+11	28	13	32	32	12
End of stage I.	+20	35	22	83	83	28
Middle stage II.	+26	35	28	90	90	30
End of stage II.	+36	35	31	92	92	30
Middle stage III.	+40	35	± 30	± 180	± 100	30
End of stage III.	+57	35	± 68	350-500	100-250	30

Together with a few adjacent cells of the pericarp which are similar in shape and orientation, they form a band or "hoop" of transversely elongated cells bounding the inner ovary wall. Since they lie at right angles to the other cells of the stony pericarp, the boundary between the two is sharp and makes a clear division between the sections which at maturity are designated inner stony pericarp and outer stony pericarp.

TSCHERSKE (19) pictures a similar condition in the pericarp of *Potentilla anserina*; and LAMPE (11), WINTON (24), and FARMER (8) observed an identical situation in species of *Rubus*. FARMER records "a chain of such fibres running around the fruit cavity resembles a segmented ring or hoop . . . and the whole of the interior of the ovary appears to be bounded by these 'rings' which run in a transverse direction round it. . . . the rest consists of fibres. . . . whose longest axis runs in a direction at right angles to that of the cells just described. The result of this arrangement is that the ovarian cavity is protected by a band of tissue, of which one half is fitted to stand strains and stresses in one direction, while the other half is equally well capable of resisting similar strains and stresses in a direction at right angles with the first" (8).

By rapid division within the tissue which is to become the stony pericarp, the number of cells is nearly trebled by the time of full bloom. The diameter increase of cells is, however, only 10 per cent. By the time of full bloom the tissue is well defined as a region of small compact cells scarcely half the diameter of the adjacent cells of the fleshy pericarp. The inner stony pericarp is approximately four cells thick and the outer stony pericarp ten cells thick.

It may be well to emphasize that the groups of cells which give rise to the stony pericarp apparently do not give rise to cells of the fleshy pericarp, or vice versa. It is not a case of the entire pericarp being derived from a common group of cells, some of which toward the center of the fruit become sclerenchymatous and some of which toward the periphery remain parenchymatous and enlarge. Nor is it a case of progressive hardening of cells of a common tissue from the center of the fruit outward in a radial direction and the enlargement of the remaining unhardened cells to comprise the fleshy pericarp. On the contrary, the two tissues appear to be derived each from dis-

tinct groups of cells which are early separated by characteristic size, shape, and frequency and periodicity of division. There is no transition in size from the smaller cells of the tissue of the stony pericarp to the larger cells of the fleshy pericarp. Moreover, mitotic figures are relatively more abundant in the tissue of the stony pericarp at the time that greatest increase in number of cells by actual count occurs, and a similar condition obtains in the tissue of the fleshy pericarp.

STAGE I.—During stage I (fig. 3*C, D, E*) the total thickness of the wall of the stony pericarp at the cheek increases approximately five times. This is brought about by a large increase in both number and size of cells. Enlargement of cells for both the inner and outer sections is marked,—an increase of approximately sixteen times in length and two or three times in width. In the inner layer, elongation takes place in a tangential direction, as seen in cross section, but in the outer layer elongation is in the direction of the central axis of the fruit. Scattered groups of cells may be found which are oriented in planes at variance with the general pattern.

By the end of the period the number of cells along the radius of the pit at the cheek has increased from fourteen to twenty-four, the greatest increase occurring in the outer stony pericarp. Active cell division continues later than in the fleshy pericarp. In the inner stony pericarp no cell divisions have been observed after the middle of stage I, but in the outer stony pericarp the greater increase occurs in the latter half. Likewise the increase in the size of all cells takes place largely in the latter half of the period (fig. 3*E*). By the middle of stage I the cells have increased from 10 to 12 μ in transverse diameter, while at the end of stage I they measure 28 μ .

Cell walls become progressively thicker but do not harden appreciably until near the end of the period. At the end of the stage the pit is too hard to section without special treatment, although the walls have not yet attained more than one-fifth the thickness found in the ripe fruit.

STAGE II.—Thickening and hardening of the cell walls of the stony pericarp are the most characteristic developments of stage II (fig. 3*E, F, G*). Although maturation of the tissue is progressing rapidly as evidenced by thickening and hardening of the cell walls, yet ac-

tive cell division occurs here as late as, if not later than, in any other part of the pericarp. This may be explained by the fact that hardening does not occur uniformly throughout the stony pericarp. It begins near the apical end, at the hilum, and extends downward along the ventral sutures toward the chalaza, before finally including the entire stony pericarp. These observations agree fairly completely with those of MIKI (16) and RAGLAND (17) for the peach, and BRADBURY (4) for the sour cherry. At the completion of the period the stony pericarp is composed of twenty-six to twenty-seven layers of very thick-walled, lignified cells. This is nearly half the total number of layers in the entire pericarp, but because of the much smaller size of the cells of the stony pericarp, they represent much less than half the thickness of the ovary wall.

No cell divisions have been observed in this region beyond the middle of stage II. The cells are living, however. Nuclei are abundant, and by their bright reaction to safranin suggest activity. Such suggested activity would seem in agreement with the tremendous thickening of the walls and their impregnation with materials. The lumen is frequently scarcely larger than the nucleus.

STAGE III. During stage III (fig. 3*G, H, I*) there is slight increase in hardness and brittleness of the tissue, associated with loss of moisture (15). Although by the time of fruit ripening most of the cells have become matured as sclerenchyma, it is still possible to find occasional living cells with active nuclei.

FLESHY PERICARP

PRE-BLOOM. Eighteen days before full bloom the fleshy pericarp is composed of nearly isodiametric, parenchymatous cells (fig. 4*A, B*). There is a suggestion of tangential elongation near the epidermis and a gradual change in orientation to a slight radial elongation near the stony pericarp. Increase in the thickness of the tissue, which is a little more than doubled, is due mostly to cell division, although there is some very slight cell enlargement. In a radial direction through the cheek, from the epidermis to the stony pericarp, the cells increase approximately 40 per cent in number during the period.

STAGE I.—During stage I the number of cells across the cheek from epidermis to stony pericarp is increased 20–30 per cent, most

of the increase occurring during the first half of the period. No mitotic figures could be found after the tenth day from full bloom. ADDOMS, NIGHTINGALE, and BLAKE (1) and RAGLAND (17) have noted a similar rapid cell division in the peach following full bloom, and its cessation early in stage I; and TETLEY (18) has found that growth in the apple after the time of fruit set is mostly a matter of cell enlargement.

Cell diameter is quadrupled, from 20 to 83 μ , the increase occurring mostly in the latter half of the period. Intercellular spaces become numerous and prominent toward the end of the period.

A hypodermal layer five or six cells thick is differentiated just beneath the outer epidermis during this period, by considerable thickening of the cell walls (fig. 5*D*, *E*). The cells attain but little greater size than those of the epidermis, but become somewhat elongated tangentially as seen in cross section. The shape of the cells of the thin-walled parenchyma remains essentially the same throughout the period, except in the outer portion where some tangential elongation takes place (fig. 4*C*, *D*, *E*).

A layer of small isodiametric cells three or four cells wide is formed adjacent to the stony pericarp during the latter part of the period. With the possible exception of this layer, there is no further cell division in the fleshy pericarp after completion of stage I. The combined activity of cell division and enlargement throughout the fleshy pericarp results in an increase in thickness of seven times. Nearly the same figure applies to the stony pericarp; hence the growth is fairly uniform for both tissues. Cell division occurs later in the stony than in the fleshy pericarp, notwithstanding the earlier differentiation of the former (fig. 4*E*, *F*, *G*).

STAGE II.—During stage II there is some small enlargement of the cells of the fleshy pericarp, from a diameter of 83 to 92 μ . Tangential elongation of the cells just beneath the outer epidermis continues slowly. Intercellular spaces remain prominent. There may be some cell division in the thin layer of small cells adjacent to the stony pericarp, but otherwise there is none. Increase in thickness of the fleshy pericarp is not more than 6 per cent.

STAGE III.—During stage III, the final swell (fig. 4*G*, *H*, *I*), four divisions of tissue may be recognized, exclusive of the outer epider-

mis: (a) the hypodermal layer of collenchyma; (b) a peripheral layer of thin-walled parenchyma extending from the hypodermal layer to a line just inside the ring of vascular bundles; (c) a layer of radially elongated cells extending from this line nearly to the pit; and (d) a thin layer of small cells adjacent to the pit.

While for convenience an arbitrary classification of tissues has been made, it must be realized that there is a gradual transition from a tissue in which one type of cells predominates to that in which another is dominant. As seen in some sections, thin-walled cells of the fleshy pericarp may occasionally come in direct contact with the epidermis; or the layers of tangentially stretched and flattened collenchyma of the hypodermal layer may be wanting. Likewise the collenchyma gives way gradually to the three or four layers of large, more nearly roundish cells of thin-walled parenchyma. These in turn give way to radially elongated but notably widened or obovate cells, which pass to progressively more narrow, radially elongated cells toward the pit.

The chief feature of this stage, often called the final swell, is the increase in size of the individual cells of the fleshy pericarp, the largest indicating an increase of as much as twenty-five times in diameter from the size at full bloom. The cells of the hypodermal layer become enlarged in a tangential direction, similar to the adjacent cells of the outer epidermis. Those in the outer portion of the fleshy pericarp increase several times in diameter and become roundish oval in shape, with the greatest diameter parallel to the periphery of the fruit. They become progressively larger from the hypodermal layer inward to the region of the vascular bundles, and the tangential elongation becomes progressively less marked. Near the ring of vascular bundles the cells are essentially round.

In the middle region of the fleshy pericarp tremendous radial elongation occurs, an increase of four to six times, and some enlargement tangentially. The tangential enlargement is progressively less toward the stony pericarp. As would be expected from the rapid stretching, there is a decrease in the thickness of cell walls. Inter-cellular spaces, which were so frequent and conspicuous during stage II, are now difficult to find. This may be due in part to the relatively greater size of the individual cells and the attendant decreased like-

lihood for the inclusion of intercellular spaces in a cross section. Or it may be that increase in pressure within the cells accompanying enlargement causes the relatively thin walls to lie more closely together. ADDOMS, NIGHTINGALE, and BLAKE (1) found intercellular spaces much less conspicuous in the peach than in the apple, and suggested that in the case of the peach, larger areas of the cell walls are in contact with the walls of adjacent cells than in the apple. TSCHERSKE (19) observed the absence of intercellular spaces in the region of elongated cells in the fleshy pericarp of the red raspberry. At all events the nature of the tissue is one of closely compressed and compacted cells with inconspicuous intercellular spaces, a striking contrast to the description which may be found in the literature of loose-fitting cells with large intercellular spaces.

The band of three to five rows of very small isodiametric parenchyma cells which lie adjacent to the pit makes no notable increase in size during this period. The cells lie between the large radially elongated cells on the one side and the cells of the stony pericarp on the other. Although the line of demarcation between the stony and the fleshy pericarp is distinct, cells of the one are more or less dovetailed between cells of the other, so that the outer surface of the stony pericarp when separated from the fleshy pericarp is slightly rough and somewhat pitted.

The total number of rows or layers of cells in a radial direction through the cheek of the fleshy pericarp has been counted as twenty-seven to twenty-nine (table 1). It is of interest to note that LAMPE (11) gives twenty-eight as the number from observations of *Prunus acida* made in 1886. Further, the number of layers of cells is nearly equally divided between the stony pericarp and fleshy pericarp, being twenty-six to twenty-seven for the former a total of fifty-three to fifty-six.

VASCULAR BUNDLES

The ventral and dorsal carpillary bundles lie wholly without the stony pericarp, although close to it. Two bundles which supply the two ovules, called in the peach "funicular bundles" by RAGLAND (17), lie at either side of the ventral suture and adjacent to the ovarian cavity (fig. 2). There are no "pit bundles" traversing the stony pericarp in the cherry, such as he describes for the peach.

Extending through the fleshy pericarp in a direction parallel with the central axis, as seen in cross section, is a ring of vascular bundles, generally eighteen to twenty in number. They are differentiated as early as 18 days before full bloom, but the walls of the xylem elements do not become sufficiently thickened to become conspicuous until about the time of full bloom. They correspond to the ring of "pit bundles" in the stony pericarp of the peach (18).

As the fruit develops, the number of bundles in the ring is not increased. The proportion of vascular tissue to the total area of the pericarp as seen in cross section thus appears much greater in the early stages than in later ones, as shown in outline drawings (fig. 2). The vascular system, moreover, is extended progressively throughout the tissue as the fruit develops, largely by transverse divergence of smaller bundles from the main bundles, similar to the condition reported in the peach by RAGLAND. At maturity the vascular bundles ramify throughout the fleshy pericarp to give a skeleton network of conductive tissue.

Throughout the development of the fruit until just before ripening there are certain cells which are conspicuous because of the granular nature of their contents and their absorption of safranin and orange G stains. They tend to form a loose network throughout the fleshy pericarp, with greatest concentration around the vascular bundles and in a layer several cells thick just beneath the outer epidermis. They are slightly larger than the average for cells of the fleshy pericarp. In the ripe fruit the granular nature of the contents disappears and the cells become indistinguishable from the adjacent parenchyma.

EPIDERMIS

The outer epidermis is a single row of cells covered externally by a cuticle which is continuous except where interrupted by stomata. The cells of the epidermis are well differentiated in material collected 18 days before full bloom (fig. 5A). At this time they are elongated radially, being about twice as long as broad. The development is similar to that in the peach as described by DORSEY and POTTER (7), excepting that there are no hairs, of course. During the entire period they increase slowly in size, chiefly in a radial direction

(fig. 5*B*). Eighteen days before full bloom an average of 312 cells was counted in the periphery of a cross section through the cheek. Two days before full bloom this number had increased to 390 cells.

During stage I (fig. 5*C, D, E*) the epidermal cells increase decidedly in both number and size. At 1 day after full bloom, 550 cells were counted in the circumference of a cross section. At the end of stage I this number had reached slightly more than double, 1187. The increase in numbers takes place very rapidly in the first 10 days of the stage; no divisions were observed in the latter part. Cell enlargement in both radial and tangential diameters occurs mostly toward the end of the period. The cuticle attains full thickness during the latter part of the period, and the radial walls are considerably thickened.

During stage II (fig. 5*E, F, G*) development of the epidermis consists in a slight tangential elongation of the cells and additional thickening of the walls. No cell divisions occur.

During stage III (fig. 5*G, H, I*) there is great enlargement of the epidermal cells in tangential directions, accompanying the large increase in the surface area of the fruit. Since cell division ceases during stage I, any increase in the size of the fruit must be compensated for by a tangential stretching of the epidermal cells. Actually the tangential diameter is more than doubled during this period, while there is a slight decrease in the radial diameter as well as in the thickness of the cuticle. The drawings (fig. 5*I*) are similar to those by DORSEY and POTTER (7) for the peach, and KOSEMANOFF (10) for the sweet cherry.

STOMATA.—The stomata are unevenly distributed over the surface of the cherry. They are numerous near the apex and few near the stem end. By 18 days before full bloom they are fully differentiated (fig. 6*A*).

Since the epidermal cells increase materially in number during later stages and no more stomata are formed, they appear relatively numerous and close together in this early stage. The guard cells increase in size as the fruit develops (fig. 6*B, C*) but the increase is much less than that of typical epidermal cells. At 18 days before full bloom the guard cells measure 13 μ in length; at full bloom, 23 μ ; during the middle of stage I, 25 μ ; at the end of stage I, 31 μ ; during

the middle of stage II and at the end of stage II, $34\ \mu$; at the end of stage III, $47\text{--}49\ \mu$.

During all stages from the middle of stage I to fruit ripening, the walls of the cells adjacent to the guard cells are very much thinner than those of typical epidermal cells. In the ripe fruit there is no development of chromoplasts in the guard cells nor in the cells immediately adjacent. In fresh unstained material the cell contents appear yellowish or straw-colored, in contrast to the bright red color of epidermal cells well supplied with chromoplasts.

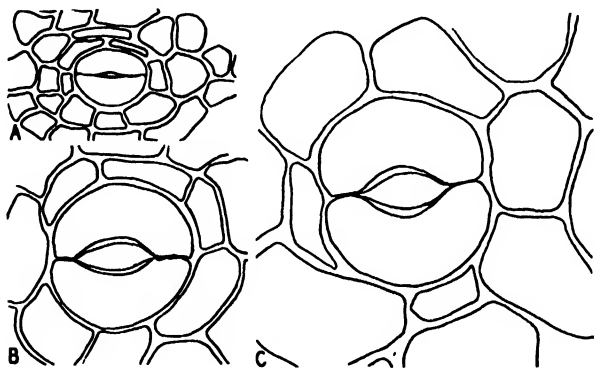


FIG. 6.—Development of stomata, guard cells, and adjacent epidermal cells in surface view (camera lucida $\times 480$). A: 18 days before full bloom; B: 20 days after full bloom; C: fruit ripening, 57 days after full bloom.

The general appearance resulting from these areas of contrasting colors is of dots which may be easily seen with the naked eye, and suggests lenticels. DORSEY and POTTER (7) described a similar appearance in the peach. No true lenticels have been observed in the material studied. Occasional rupture of the epidermis from mechanical causes has been seen; and discolored areas were found, the result of spray materials or some mechanical injury. Even in ripe fruit which had been allowed to remain on the trees until September 20, 60 days after commercial harvest, stomata and guard cells were still intact (fig. 6C) and no lenticels were found.

Discussion

The shape of the cells of the fleshy pericarp from the periphery of the fruit to the stony pericarp, as seen in cross section, appears

much as it might if each cell was subjected to relatively equal internal force of expansion in all directions, such as osmotic pressure.

The cells nearest the pit can expand very little except in a radial direction, because of the close position tangentially of other cells exerting similar pressures toward them. The cells surrounding the first ones, besides making a radial elongation, can now expand slightly in a tangential direction, since the diameter of the fruit has become greater and the distance in a tangential direction between the theoretical centers of the rows of cells has become greater, similar to the increase in distance between the spokes of a wheel the farther from the hub. Progressively toward the periphery of the fruit the tangential pressure from adjacent cells becomes less, so that the cells assume an obovate shape, with the small end pointing toward the center. Finally, near the periphery of the fruit the internal cell pressure is equally balanced by the inward pressure of the stretching epidermis and hypodermal layer, by the outward pressure of inner cells, and by the tangential pressure of adjacent cells, so that they assume a roundish shape. In some instances these cells may even be broadly oval. The cells of the hypodermal layer and epidermis, being subjected to outward pressure from the expanding thin-walled parenchyma, become stretched tangentially and flattened.

In figure 7 is shown a series of camera lucida drawings of the cells in a radial sector of the ripe fruit from the stony pericarp to the outer epidermis, illustrating how closely the cell shapes and sizes conform to the preceding description. ADDOMS, NIGHTINGALE, and BLAKE (1) have remarked upon the radial elongation of cells of the fleshy pericarp of the peach near the pit and the more ovoid shape further out.

The gross size changes in the fruit during the three stages of development are seen from this study to be made up of cell divisions and cell enlargements in varying proportions in different tissues at different times. Figure 8 shows the amount of increase in a transverse direction through the wall of the fruit at the cheek. It has been prepared by calculation from the actual numbers and sizes of cells in the different tissues at the beginning, middle, and end of each growth stage (tables 1 and 2). While the general nature of the curve is similar to that for the total diameter of the fruit in figure 1, it differs in

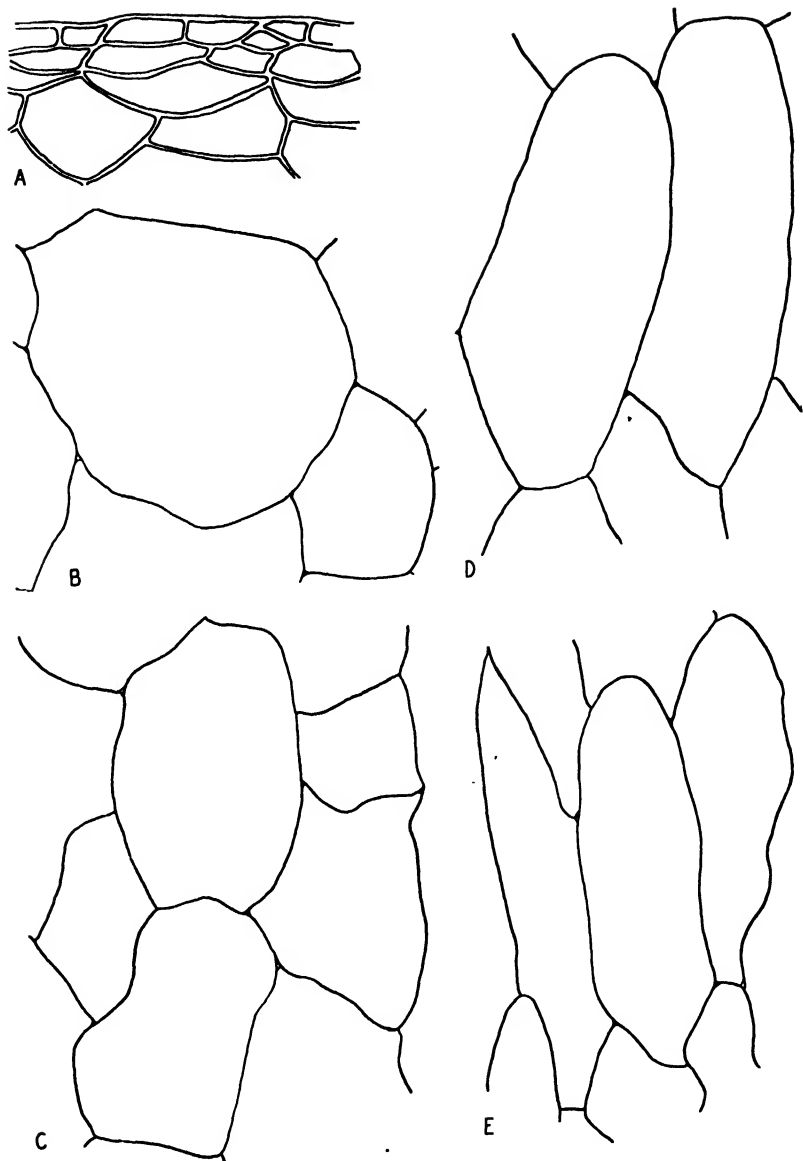


FIG. 7.—Size and shape of cells involved in final swell of fleshy pericarp in radial direction through cheek of sour cherry at fruit ripening (end of stage III), showing transverse elongation in epidermis and hypodermal layer (A); roundish shape in next underlying region between hypodermal layer and ring of vascular bundles (B); oval shape and beginning of radial elongation in next underlying region (C); long oval shape in next underlying region (D); and decided radial elongation in inner region (E). Inmost layer of small cells adjacent to stony pericarp not shown (camera lucida $\times 140$).

that it represents only the actual increase of the tissues of the ovary wall, whereas figure 1 also includes the ovarian cavity besides both sides of the ovary wall. Since the increase in the diameter of the ovarian cavity during stage I does not involve a corresponding increase in tissue, the steepness of the curve in stage I of figure 8 is much less than that of figure 1. The figure does show graphically the relation between cell division and cell enlargement and the part played by both in the growth of the cherry fruit.

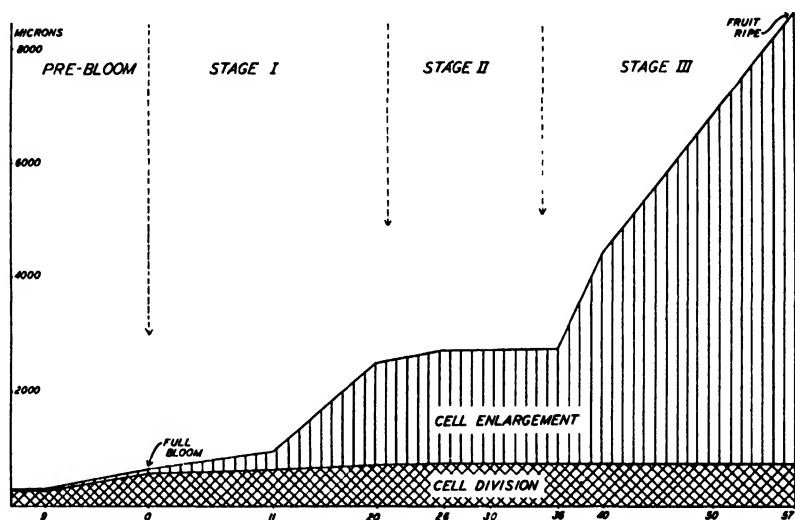


FIG. 8.—Increase in thickness of ovary wall of sour cherry at cheek, from pre-bloom to fruit ripening, showing amount due to increase in number of cells and amount due to enlargement of cells already present. Vertical lines=size changes due to cell enlargement. Cross hatching=size changes due to cell division.

The similarity of development of the sour cherry to the development of other drupe fruits has been noted. The studies of TSCHERSKE (19) with the red raspberry are particularly noteworthy. He observed two periods of rapid development in the drupelets: a first one early in the season during which cell division mainly occurs, and a second just before fruit ripening, in which stretching of the cells occurs. Particularly interesting is his observation that the final swell begins when the structures of the seed and surrounding tissues are differentiated, as noted for the cherry and peach by TUKEY (21, 22).

At maturity the fruit bears a close resemblance to the descriptions given by TSCHERSKE (19) for the drupelets of *Rubus ideaus*, by FARMER (8) for *R. fruticosus*, and by WINTON (24) for *R. strigosus* and *R. occidentalis*. Further, WINTON mentions a striking similarity between the red raspberry and the peach.

Summary

1. This paper pictures and discusses the gross development of the fruit of the sour cherry (*Prunus cerasus* L. var. Montmorency) from 18 days before full bloom to fruit ripening, and the histological changes during the pre-bloom stage, stage I (rapid development for 20 days following full bloom), stage II (retarded development for 16 days), and stage III (rapid development for 21 days to fruit ripening).

2. Three principal tissues compose the ovary wall: inner and outer epidermis, stony pericarp, and fleshy pericarp. The stony pericarp may be divided into an inner and an outer layer, and the fleshy pericarp into an innermost layer of small thin-walled parenchyma, a middle region of large thin-walled parenchyma, and an outer or hypodermal layer of collenchyma.

3. The fleshy and the stony pericarp are derived each from distinct groups of cells which are early separated from one another by characteristic size, shape, and frequency and periodicity of cell division.

4. The inner layer of the stony pericarp is derived from the inner epidermis, together with a few adjacent cells of the pericarp, and forms a band or "hoop" of transversely elongated cells bounding the inner ovary wall. The outer layer is derived from the pericarp, and the cells of which it is composed are elongated at right angles to those of the inner layer.

5. The cells of the stony pericarp increase in number during the pre-bloom stage and the first few days of stage I, and enlarge during the latter part of stage I. Cell walls become progressively thicker, and by the end of the period the maximum number and size of cells is attained. During stage II the walls thicken and harden greatly. During stage III there is slight increase in hardness and brittleness.

6. The cells of the fleshy pericarp increase in number during the pre-bloom stage and the first half of stage II. In the last half of stage II they double in diameter. During stage II there is slight enlargement. During stage III the size of individual cells increases remarkably. Those of the hypodermal layer become enlarged in a tangential direction similar to the adjacent cells of the outer epidermis. Those in the outer portion of the fleshy pericarp become roundish oval, with the greatest diameter parallel to the periphery of the fruit; those next inward become roundish; those next, obovate in a radial direction; next, radially elongate; and innermost, decidedly radially elongate. At maturity the largest cells indicate an increase of twenty-five times in diameter from the size at full bloom.

7. The epidermal cells are elongated radially 18 days before full bloom. They increase rapidly in number during the pre-bloom stage and during the first half of stage I, increase in size and wall thickness during the latter half of stage I, change but little during stage II, and greatly enlarge tangentially in stage III.

8. The stomata are fully differentiated 18 days before full bloom. The guard cells increase in size as the fruit develops, but the increase is less than that of typical epidermal cells.

9. The similarity of other fruits and the mechanism of enlargement in stage III are discussed.

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PLANT SUCCESSION ON GRANITE ROCK IN EASTERN NORTH CAROLINA

HENRY J. OOSTING AND LEWIS E. ANDERSON

(WITH NINE FIGURES)

Introduction

A few studies of the development of vegetation upon bare rock have been made in widely separated sections of the country. These show that the progression of growth forms and even of certain genera is remarkably similar everywhere. Following our study¹ of a mountain exposure in North Carolina, it seemed logical to make a comparative study of lowland outcrops. Several outcrops were located on or very near the fall line which marks the transition between the Coastal Plain and Piedmont provinces.

The transition between the Coastal Plain and the lower Piedmont is usually gradual, but there is a definite change in level. Along the fall line the higher lying rock of the Piedmont is frequently exposed in areas of varying sizes up to several acres. In North Carolina these outcrops are most abundant and extensive northeast of Raleigh in Wake and Franklin counties, but lesser ones appear intermittently toward the southwest. In a comparable physiographic position in South Carolina there are extensive outcrops in Lancaster County, and in Georgia they are both numerous and widespread.

The rock is granitic and weathers slowly. The surface is smooth and usually free of crevices, while the general relief is interrupted only by minor depressions and irregularities (figs. 7, 9). Several areas are crossed by streams and others have a slope sufficient to cause vigorous washing by heavy rain.

The annual rainfall for the region varies from 45 to 46 inches, most of the precipitation occurring in winter and spring. Summer is characteristically hot, with extended dry periods. The resulting forest types are typical of a large portion of the southeastern states. Oak-hickory forests occupy old undisturbed sites, while loblolly

¹ OOSTING, H. J., and ANDERSON, L. E., The vegetation of a barefaced cliff in western North Carolina. *Ecology* 18:280-292. 1937.

(*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) grow in abandoned fields and cutover land. The poorer sites support Virginia pine (*P. virginiana* Mill.) or post oak (*Quercus stellata* Wang.) and black jack oak (*Q. marilandica* Muench.). The latter are the usual species near the outcrops, indicating that the soil mantle is probably thin for some distance around the exposed rock.

Conditions for plant growth on exposed rock are obviously extreme. The habitat requires adaptations, resulting in a flora that is duplicated nowhere else, and many species of plants so adapted are found growing only on rock. Their ranges are accordingly determined by the geographical occurrence of the rock exposures, and for these reasons may well be considered as rock endemics. The hot dry summers of the southeastern states further accentuate the factors controlling rock vegetation, and result in an especially large number of rock endemics for the section.

The richest flora is found in early spring when temperature and moisture are most favorable. Several species appear then in great abundance, but with the coming of summer they soon disappear and the entire aspect changes. Only the true dominants remain. Especially in the early stages of succession the dominant species are few. All are low growing and mat-forming.

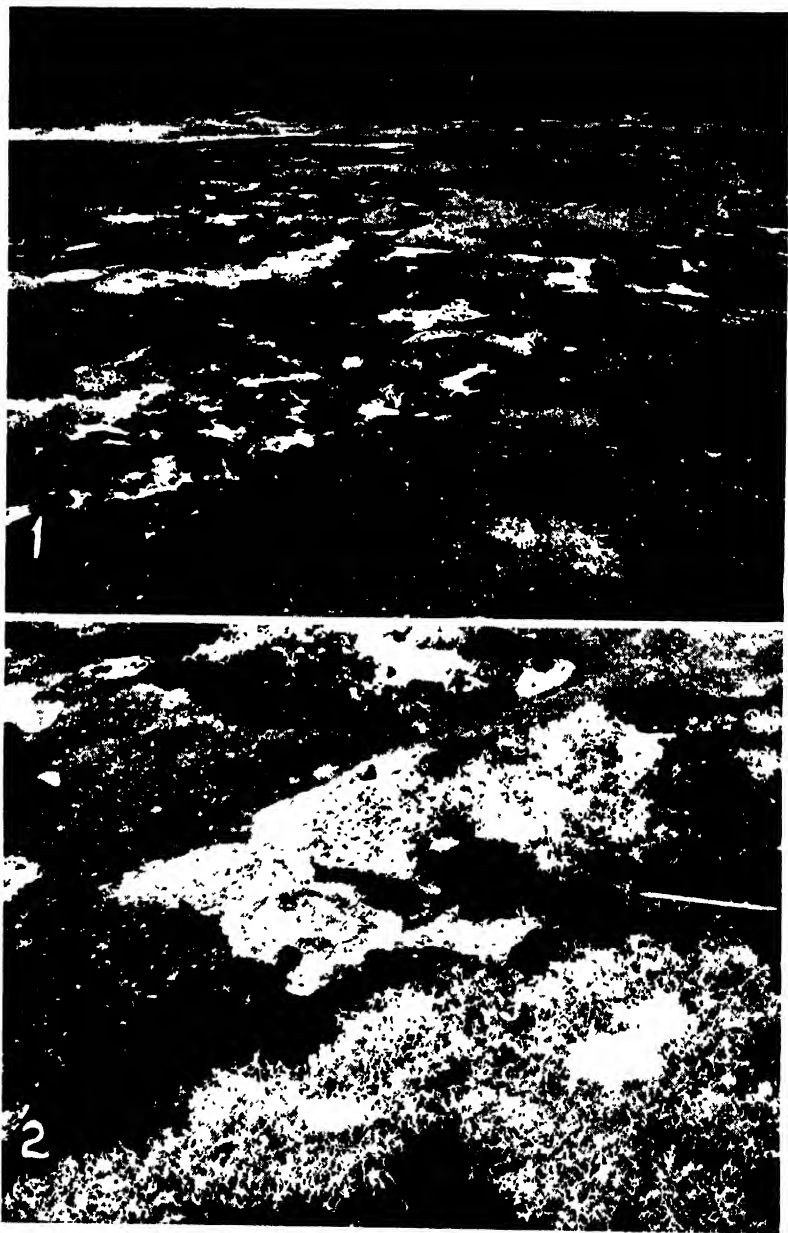
Such a widely extending special habitat, capable of supporting numerous species with restricted ranges, deserves further botanical consideration.

Succession

It is necessary to distinguish two surface variations which determine succession upon the bare rock. Each has a characteristic vegetational development and each materially affects the rate of change. These variations are (1) the dry bare rock surface in general, and (2) the depressions below the level of the surface proper.

ROCK SURFACE

Pioneers may appear wherever a minute crevice or roughened surface allows the plants an anchorage. Whether the surface is flat, undulating, or steep (as on the slopes of some of the domed outcrops), the vegetation is much the same everywhere. Moisture can be retained only by mat-forming species and then only for a short



FIGS. 1, 2.— Fig. 1, pioneer stages of mat formation on bare rock, showing mats of *Grimmia* and of *Grimmia* and *Cladonia*. Fig. 2, detail of *Grimmia* spreading on bare rock with *Cladonia* invading the mat. Note small patches of former beginning on bare rock.

time. The pioneers may be desiccated for long periods at any season of the year. Change is extremely slow and in some places may actually be in a condition of pioneer equilibrium.

The earliest pioneers are uniformly the same on every rock area studied, regardless of the surface of the rock. *Grimmia laevigata* (Brid.) Brid. is regularly the pioneer mat former (fig. 1). Its habitation of the rock is definitely not restricted to areas occupied by crustose lichens or by other previous invaders (figs. 1, 2). Crustose lichens such as *Verrucaria nigrescens* Pers. are perhaps the first plant occupants, but they accumulate so little soil as to be of no importance in aiding later plants to become established. This is also true of *Parmelia conspersa* (Ehrh.) Ach., a foliose form which, although present in restricted patches on all the rock, never shows evidence of being replaced by other species (figs. 2, 4).

Small tufts of *Grimmia*, once established, spread apparently by vegetative means and may form mats of considerable extent before being invaded by other species (fig. 1). These mats of *Grimmia* catch and retain a comparatively large amount of mineral soil which blows or washes over them. Soil often accumulates until only the tips of the plants are visible and the mats may be 1 to 3 inches deep. Associated with *Grimmia* when considerable mineral soil is held in the mats is the small succulent *Diamorpha cymosa* (Nutt.) Britton. Although in spring it may occur so thickly as to obscure the *Grimmia*, it disappears in summer and is relatively unimportant in aiding further development of the mats. *Diamorpha* may be considered merely as an associate of *Grimmia* growing in the mineral soil in a situation free from competition with larger species which are unable to survive in such severe conditions.

The pioneers at the periphery of a mature mat are continually spreading upon the rock. At the same time new species are invading the center of the mat, which is the oldest portion. These invaders advance centrifugally over the mat at about the same rate that the pioneers spread upon the rock, and they may themselves be superseded by other species which again invade the central area. This results in a series of more or less concentric girdles, each representing a stage in mat development. The pioneer stage is invariably at the periphery, the most mature at the center. There are numerous

species associated with these stages, but their relationships are clear when considered in the rather distinct girdles. Each girdle usually has but a single dominant. The other species are seasonally important or may merely be associated with the dominant because of the conditions it provides. The dependent species contribute little to the development of the mats.

Successional development of a *Grimmia* mat comes through invasion by *Cladonia leporina* E. Fries and rarely by *C. caroliniana* Tuck. (fig. 2).² The lichens spread from the center of the mat at about the same rate that *Grimmia* is advancing. The lichens, being coarse and large, add considerably to the thickness of the mat, facilitating the collection of soil. At the same time or shortly after the appearance of *Cladonia*, *Selaginella rupestris* (L.) Spring invades the mat, whereupon mixed growths of *Cladonia leporina* and *Selaginella rupestris* result (fig. 3c). These species, being coarser and much larger than *Grimmia*, contribute far more humus and offer a greater resistance to wind- and water-borne debris. Thus with their invasion the rate of succession is accelerated. *Arenaria brevifolia* Nutt., especially conspicuous in spring (fig. 4), is associated with this girdle. When desiccation begins it entirely disappears. Although it may be present on *Grimmia* mats, it reaches its maximum development with the *Selaginella* stage.

With the deepening of the mat due to both mineral and organic additions, *Polytrichum ohioense* Ren. & Card. makes its appearance (fig. 3d). *Selaginella* and *Cladonia* are soon completely excluded. Here again are conspicuous associates which probably affect the mat only slightly. *Crotonopsis elliptica* Willd. and *Hypericum gentianoides* (L.) BSP., although growing elsewhere in some abundance, are here consistently present and evenly distributed.

Conditions for growth are now apparently comparable with those of some abandoned fields in the vicinity, for a weed complex appears that is similar to the vegetation in fields during the early years of their abandonment. In addition to *Hypericum*, *Diodia teres* Walt., *Ambrosia artemisiifolia* L., and *Panicum philadelphicum* Bernh. are

² We acknowledge with thanks the cooperation of Dr. ALEXANDER W. EVANS, who identified the *Cladoniae* mentioned in this study.

initiates and *Geranium carolinianum* L., *Trifolium procumbens* L., *Krigia virginica* (L.) Willd., *Linaria canadensis* (L.) Dum., *Gnaphalium purpureum* L., and *Cerastium vulgatum* L. are typical later



FIG. 3.—Margin of mature mat showing successive girdles of *Grimmia*, *Cladonia*, *Cladonia* and *Selaginella*, and *Polytrichum*.

members. Eventually, as in old fields, *Andropogon virginicus* L. and *A. ternarius* Michx. become the dominants (fig. 7), with *Eupatorium hyssopifolium* L., *E. rotundifolium* L., *Senecio smallii* Britton, *Salvia lyrata* L., and *Panicum xalapense* HBK. as associates. Especial men-

tion should be made of *P. columbianum* var. *thinium* Hitchc., since it has been found nowhere in North Carolina except on these outcrops.

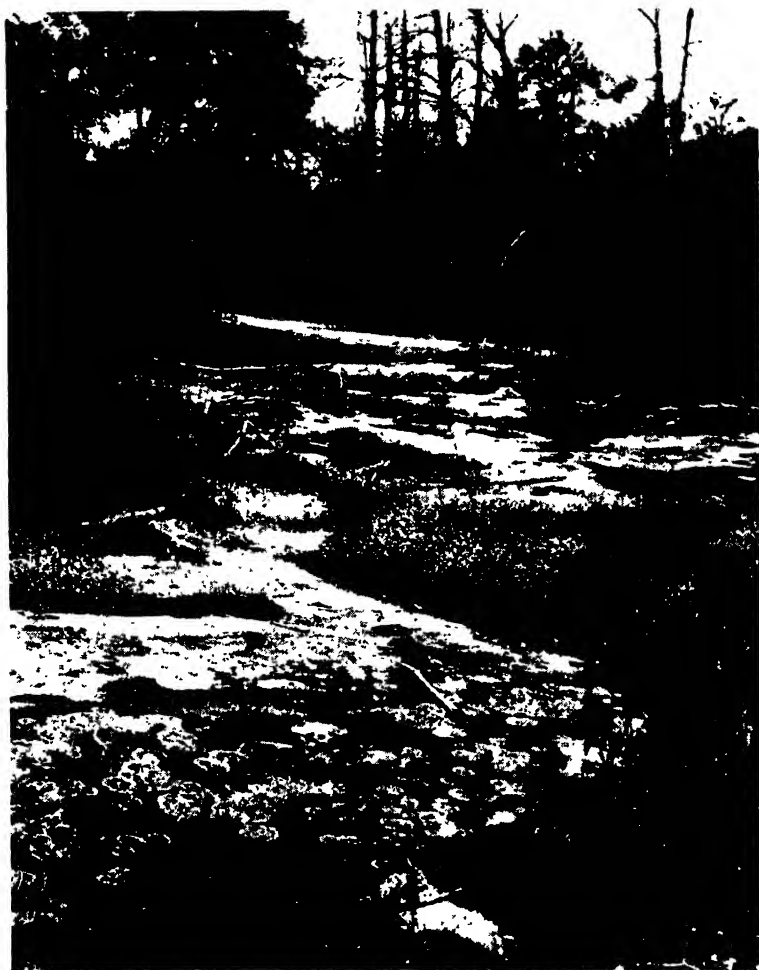


FIG. 4.—Margin of mat in spring. Girdles obscured by mass of *Arenaria*. Note large *Juniperus virginiana* on mature mat at left and dead pines in background.

If given time, such a mat thickens and develops a soil layer capable of supporting certain woody seedlings which germinate upon it. *Juniperus virginiana* L. is a typical early tree species (fig. 4),

with such drought resistant shrubs as *Rhus copallina* L. and *Baldendron arboreum* (Marsh.) Nutt. as associates (fig. 8). *Pinus virginiana* or *P. echinata* is apt to follow if seed trees are near.

The tree stage is nowhere well developed. Mats are never sufficiently extensive to support more than a few trees and some of them invariably are dead (fig. 4). The scrubby habit and slow growth rate of the woody species indicate the unfavorableness of the habitat.

DEPRESSIONS

The regularity of the rock surface is interrupted here and there by depressions, which vary in depth and size. The associated vegetational effects are caused by the water, which after every rain is retained until evaporated or used by the plants. On the basis of depth and drainage, there may be dry depressions, moist depressions, or pools, each with a distinctive vegetational development.

DRY DEPRESSIONS.—Most of the depressions are shallow and flat bottomed, the amount of water held being determined by the level of the lowest part of the rim. When a portion of a rim is eroded, the depression may retain only a thin layer of water, which in summer evaporates soon after it has fallen. The rim of the depression serves to obstruct and retain transported soil, which forms a level floor of silt and sand that bakes hard and dry after every rain. Such depressions are perhaps less favorable habitats than the bare rock itself. The layer of soil is too thin and bakes too hard to support most species, while its depth excludes the bare rock pioneers. Only succulents or small wiry species seem capable of growing in this extreme situation (fig. 7). Most of the latter are seen only in spring, leaving the flats almost completely bare in summer. *Talinum teretifolium* Pursh. is rather regularly present, as is *Portulaca smallii* Wilson. If the soil is sufficiently deep *Diamorpha cymosa* is often abundant. Mineral soil and absence of competition seem to constitute the requirements for these rock succulents. Any of them, and especially *Diamorpha*, occur as associates of *Grimmia* when an appreciable mineral layer has been formed. *Arenaria brevifolia*, a delicate annual growing only in early spring, is a rock species which, like *Diamorpha*, requires a mineral soil of a favorable depth and a minimum of com-



FIG. 5.--Shaded tongue of rock at edge of woods, with *Entodon* growing on organic debris.

petition. Here and there a sandy hollow may produce a solid stand of *Arenaria*, which dies as the depression dries out.

Other non-succulents surviving on the baked soil are *Fimbristylis baldwiniana* (Schult.) Torr., *F. autumnalis* (L.) R. & S., *Stenophyllus*

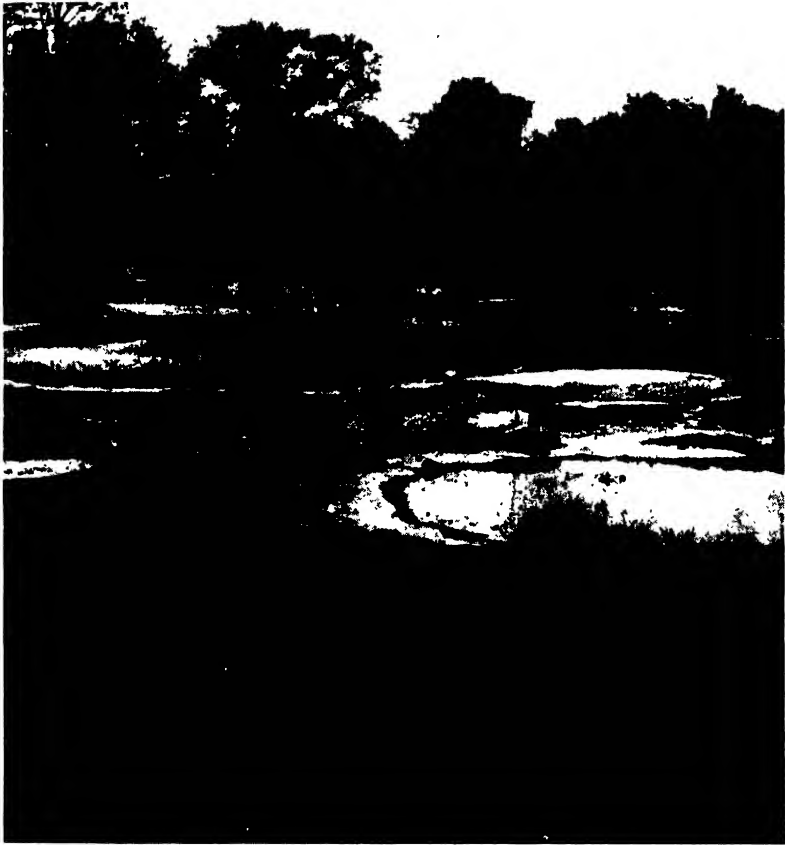


FIG. 6.—Rock pool with characteristic raised ring of pioneers around margin. Behind pool, a moist depression approaching *Andropogon* stage.

capillaris (L.) Britton, *Cyperus inflexus* Muhl., and *Agrostis eliottiana* Schult., the last being a southern species restricted to these rock outcrops in North Carolina. Such dry depressions usually give no evidence of further successional development. It is probable that many have long been in this condition and will so remain unless the contours of the marginal rock change. Others, comparable except

for a slightly deeper layer of soil, may be occupied by masses of *Diodia teres*, *Crotonopsis elliptica*, *Hypericum gentianoides*, and even a few clumps of *Polytrichum commune*. *Lechea racemulosa* Lam. with *Eupatorium pinnatifidum* Ell. represent an intermediate stage in deep sandy depressions. Indicative of the better moisture conditions is the *Andropogon* stage which follows and the fact that the shrubs include not only *Rhus copallina* and *Batodendron arboreum*, but also scattered plants of *Rubus* spp. In these deeper depressions filled with mineral soil the mature stages frequently include seedlings of *Liquidambar styraciflua* L., and still older areas which may have had a similar origin support *Pinus taeda* and an occasional *Quercus stellata* (fig. 9).

MOIST DEPRESSIONS.—Moist depressions are intermediate as to moisture, and retain some water after every rain but not enough to support species with aquatic or marsh characteristics.

Succession in the undrained depressions is less obvious and more irregular than on bare rock. The depth of water varies with the depth of the hollow and fluctuates with the supply and rate of evaporation. Few species can endure alternate periods of complete inundation and prolonged desiccation. This is the situation in most of the depressions, for few are sufficiently large or deep to be classed as permanent pools.

As elsewhere on the rock, *Grimmia laevigata* is the pioneer in the moist hollows. Depending apparently upon moisture conditions, it is followed by *Cladonia leporina*, *C. caroliniana*, or *C. tenuis* (Floerke) Harm. Occasionally all three species may simultaneously invade a mat of *Grimmia*. These species retain soil and debris efficiently and soil accumulates much faster in the depressions than on the flat surfaces already described. The accumulation of soil and the moist condition facilitate the growth of *Polytrichum ohioense*, which next invades the mat. In the moister mats *Selaginella* is not present, for it either cannot withstand the moist conditions or it is excluded by the luxurious growth of *Polytrichum*. Large tufts of *Campylopus introflexus* (Hedw.) Brid. are at times mixed with *Polytrichum*. When the moisture conditions are especially favorable the *Polytrichum-Cladonia-Grimmia* mats often attain depths of 6 to 10 or more inches.



FIGS. 7-9.—Fig. 7, general view of typical flat exposure. Moist depression in *Andropogon* stage (center and right foreground) and dry, sand-floored depression (left foreground) supporting *Stenophyllus*, *Talinum*, and *Portulaca*. Fig. 8, mat in shrub stage. *Rhus copallina* in center surrounded by girdle of *Andropogon*, then *Polytrichum* obscured by *Crotonopsis* and *Hypericum*; at very margin, advancing girdles of *Cladonia* and *Grimmia*. Fig. 9, old mat of depression origin supporting pines and hard-woods. Several trees dead.

After the early mat-forming stages, the moist hollow succession soon becomes rather like that on bare rock. The mat spreading over the rock at the margins of the depression has, of course, the same characteristics as one originating on the bare rock. The portion of the mat over the depression is thicker than a bare rock mat and has other species because of better moisture conditions. Added species, typical of mats originating in depressions include *Rumex verticillatus* L., *Sphenopholis obtusata* (Michx.) Scribn., *Panicum oricola* Hitchc. and Chase, *Galium claytoni* Michx., *Trisetum pennsylvanicum* (L.) BSP., *Myosotis virginica* (L.) Link, and *Specularia biflora* (R. & P.) F. & M. The two lines grade into each other, therefore, and the central portion of a mature mat must be examined to identify its origin. An abundance of *Rubus* spp. in the early shrubby condition and the regular presence of *Ulmus alata* and *Liquidambar styraciflua* offer rather positive evidence of depression origin. Except for minor variations the species and their relationships are much the same in the later stages as those described for bare rock.

POOLS.—Any depression which supports aquatic or marsh species may be termed a pool. Except after prolonged drought, there will always be mud in the bottom, if not standing water. Few pools are sufficiently large or deep to be permanent.

The regular rock and depression species previously discussed develop around the margins, spreading over the rock and also advancing centripetally as the water level permits. The moisture speeds growth and frequently the mat formers are present as a raised ring almost completely circling the pool (fig. 6). The marginal ring has a development comparable with that of damp depressions and invariably becomes dominated by *Polytrichum ohioense*. The ring is fringed at the water's edge by *Philonotis fontana* Brid., *Bryum bimum* Brid., *Climacium americanum* Brid., and *Aulacomnium palustre* Schwaegr. Toward the dry rock are two girdles comparable with rock succession. The *Polytrichum* grades into *Cladonia caroliniana*, with *Hypericum gentianoides* and *Crotonopsis elliptica* associated, and the outer margin is a *Grimmia* girdle with its usual associates of *Arenaria brevifolia* and *Diamorpha cymosa*, and the frequent addition of *Talinum teretifolium*. Obviously this is the normal or moist depression series spreading from the margin of the pool.

If there is no soil in the depression, the development of vegetation in the water is delayed until mineral and organic materials cover the bottom. Even then development is slow, because the pool periodically becomes dry and aquatic species may not survive. The shallower, mud-bottomed pools with little or no vegetation invariably support *Lindernia monticola* Muhl. and less frequently *Utricularia juncea* Vahl. Deeper pools are often filled with a dense tangle of *Proserpinaca palustris* L. If soil is accumulating, *Gratiola neglecta* Torr. may occupy the muddy margin, while *Fleocharis obtusa* (Willd.) Schultes, *Juncus effusus* L., and sometimes *Typha latifolia* L. form emergent clumps in the water. As these species increase they accelerate soil building and the habitat tends to become a marshy depression rather than an open pool (fig. 6).

The dominants are the Cyperaceae, including ten species of *Carex* and several species of *Cyperus*, none of which are constantly present. *Rhynchospora microcarpa* Baldw., *R. glomerata* (L.) Vahl., and *R. cymosa* Ell. may be found on every outcrop where pools are filling up. *Scleria pauciflora* Muhl. is frequently present, as is *Juncus acuminatus* Michx. Other species occurring rather generally are *Seriocarpus linifolius* (L.) BSP., *Lobelia nuttallii* R. & S., *Xyris ambigua* Beyr., *Ranunculus pusillus* Poir., *R. abortivus* L., and *Chaerophyllum tienturieri* Hook.

Shrubs appear only after a long period of the marsh condition and after the soil has been gradually built up to the level of the general rock surface. If the site remains fairly moist, *Alnus rugosa* (DuRoi) Spreng. may be an early woody species. The marshy condition may persist for a long time, supporting deep beds of *Polytrichum* and even *Sphagnum* long after woody species have gained a foothold. Intermediate moisture conditions are indicated by *Rubus*, followed by *Liquidambar styraciflua* and *Ulmus alata*.

MARGINAL VEGETATION

The borders of the more extensive outcrops are exceedingly irregular in outline. The vegetation along the margins is for the most part stable and shows little evidence of migrating upon the rock itself. Woody species usually extend to within a few feet of the edge of the rock.

Some margins are almost continuously moist because of seepage from the adjoining forest. In these seepage zones water accumulates in the outer layer of soil and vegetation, resulting in an especially luxuriant fringe of bryophytes and small herbs which have a characteristic boggy aspect. Here are deep beds of *Sphagnum recurvum* Beauv., *S. subsecundum* Nees., and *S. imbricatum* Kornschn., with *Climacium americanum*, *Aulacomnium palustre*, *Leucobryum glaucum* Schimp., and *Polytrichum commune* Hedw. If very moist, *Bryum bimum* and *Philonotis fontana* are included. Scattered on the bogs are *Danthonia sericea* Nutt., *Alopecurus carolinianus* Walt., *Veronica arvensis* L., *V. peregrina* L., and *Selaginella apoda* (L.) Fernald. Beyond the bog, along the mineral margin, the typical *Selaginella-Polytrichum* zone may be found to grade into *Andropogon* and shrub zones adjoining the forest.

Where irregular tongues of bare rock extend back into the forest the margin is shaded and protected from washing rains (fig. 5). Here loose organic debris from fallen branches and twigs accumulates. The organic material is soon occupied by *Entodon seductrix* (Hedw.) C. Muell., which forms a loose and insecure covering extending as far out on the rock as the overhanging branches of the trees. Permanent establishment is difficult because the *Entodon* is not anchored to the rock. Even though protected, the mat is periodically disturbed and only a narrow margin is stable enough for other plants to invade it. Some of these are *Thuidium delicatulum* (Hedw.) Mitt., *Dicranum scoparium* Hedw., *Cladonia strepsilis* (Ach.) Vanio., *Oxalis stricta* L., *Talinum teretifolium*, *Woodsia obtusa* (Spreng.) Torr., *Geranium carolinianum*, *Asplenium platyneuron* (L.) Oakes, *Cerastium vulgatum*, *Saxifraga virginensis* Michx., and occasionally large tufts of *Cladonia tenuis*. The vegetation of the shaded, protected margins may migrate to some extent, depending upon how successfully anchored the *Entodon* complex becomes. There is evidence that some of these protected spots may eventually become filled in with vegetation and thus even out the irregular outline of the margin.

Not all of the rock outcrops are closely surrounded by trees. In level country a few are bordered by cultivated fields, which must at one time have been wooded. Only on the open outcrops is there

evidence of a migration of marginal vegetation upon the rock. The succession is as described for "rock surface." The occurrence of marginal migration in the shadeless areas offers a clue to its absence in wooded areas where the margins are shaded for a large part of the day. *Grimmia*, which is the only mat pioneer, is intolerant of shade, and succession cannot be initiated along wooded margins. Since *Grimmia* grows only on dry, well drained surfaces, the frequent seepage areas of the shaded margins undoubtedly further exclude it because of excess moisture.

Discussion

The rock of the outcrops is similar in all localities. The outcrops occupy the same geographic position everywhere, so that the causes of their exposure must have been similar. Since the exposures are irregularly distributed along the fall line in at least three states, they must (at least indirectly) owe their origin to factors which produced the fall line.

The exposures have been in their present condition for a long period. Certainly if they originated with the fall line there has been sufficient time for vegetation to have developed a complete cover and to have produced a well bound soil mat over the largest of them. Although growing conditions are extreme and the rate of succession is slow, apparently a cover can develop on any of the exposures. It is not unreasonable to suppose that at one time the rocks were completely covered with at least sufficient soil to support a meager forest. With the advent of the white man, lumbering, fires, and erosion might well have exposed these areas where the rock was nearest the surface.

For some distance surrounding an exposure the rock is covered with a comparatively thin layer of soil, usually unsuited to agriculture. These poor sites may not support dense or well developed forest stands but almost invariably they are forested, indicating that in recent years man's disturbances of the rock margins have been only minor. The reasons for the lack of cover on the rock surfaces today must be sought elsewhere.

No mats are found which support more than a few individuals of tree size. These larger individuals are always inferior specimens and

frequently die before attaining much growth. Twenty-five years is a good average age for the larger trees on the mats. It is apparent that somehow the development of the mats is checked as they approach maturity.

Two factors probably tend to restrict the further development of the older mats. Since the trees are rooted only in the mats they have no substantial anchorage. The larger they become the more they are subject to wind-throw. Down trees are the rule on every outcrop. When a tree is upturned, its roots raise the mat with them and may mean almost its complete destruction. The second factor is fire. The numerous down trees, the highly organic mats, and the extremely dry condition of the vegetation during summer all provide ideal conditions for almost clean burning when a fire starts. In the general region of the outcrops summer fires are common. Fire protection is concentrated not on these worthless rock barrens but on more valuable properties. Consequently every outcrop has evidence of fairly recent fire, and invariably the area burned is reduced to a bare rock condition except where depressions hold mineral soil. Perhaps these two factors are sufficient to explain why the outcrops remain barren. Erosion, which must have played its part in their early exposure, is today of importance only on a few steep slopes or locally on a portion of an outcrop.

This is in contrast with the mountain exposures in western North Carolina. These are on steep slopes where an enlarging mat, unless well anchored, is doomed because its own weight will release it. Fires are infrequent and precipitation regular throughout the growing season. Here then, erosion and gravity are the factors which check the unlimited development of the mats.

The two types of exposures are roughly in the same latitude but separated some 3000 feet in altitude. This altitudinal difference results in a much shortened growing season in the mountains. However, temperatures are not so high, complete desiccation is rare, and almost continuous summer growth is possible because of the frequent mountain rains. In spite of these differences the growth forms, and even genera, are remarkably similar in their successional relationships. However, species are only rarely duplicated. It should be noted that in neither habitat do crustose lichens play any important

part in the development of later stages of vegetation. They are pioneer species in both climates, but rarely, if ever, are they followed by other forms. Xerophytic mosses comprise the pioneer community which, coming in on bare rock, begins mat formation and succession. The crustose lichens are merely incidental to the normal line of development, neither hindering nor contributing to it.

Summary

1. The transition between Piedmont and Coastal Plain provinces, called the fall line, has numerous outcrops of granitic rock scattered along its length throughout North Carolina, South Carolina, and Georgia.

2. Rocks are unfavorable habitats for plant colonization and these are particularly so because of the long dry summers with high temperatures. Especially adapted plants, including several rock endemics, grow here in spite of the extreme conditions. Succession on the North Carolina rocks follows two major lines, originating (1) anywhere on the rock surface and (2) in depressions. The depressions may be dry, moist, or pools.

3. The bare rock surface is invaded by *Grimmia* or crustose lichens. The latter do not contribute to further succession. *Grimmia* forms mats which are successively invaded by stages dominated by (1) *Cladonia-Selaginella*, (2) *Polytrichum*, (3) *Andropogon*, (4) conifers.

4. Dry depressions retain little water and are floored with a clayey soil. The soil is too thin to support species which lead to a successional series and too deep to permit the beginnings of a normal rock succession. They support scattered succulents like *Talinum* and *Portulaca*, and sometimes dry, fibrous individuals like *Fimbristylis* and *Stenophyllus*.

5. Damp depressions represent the best development on the rocks. The stages are essentially like those on the rock surface but the additional moisture results in richer and more rapid growth, with hardwoods appearing in the later stages.

6. Pools are usually intermittent but regularly support marsh species. The margins have the normal rock series spreading from the pool and Cyperaceae predominate among the soil builders in the

water or mud. Late stages are dominated first by mesophytic shrubs and later by hardwoods.

7. Margins are irregular. Where seepage occurs there may be small boggy areas dominated by *Sphagnum*. Dry margins are usually fixed and rarely show evidence of migration upon the rocks. Shaded margins, protected from washing, produce a distinctive pioneer community upon the debris which accumulates. *Entodon* is the dominant.

8. The exposures must owe their origin to factors which caused the fall line. Considering the time since this line was formed and the rate of plant succession, it is proposed that the rocks were once entirely covered although probably not so richly forested as the surrounding country. The activities of man resulted in repeated fires and erosion which again uncovered the rock, and today drought, wind-throw, and fire undoubtedly maintain them in their semi-barren condition.

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STRUCTURE OF SOME CARBONIFEROUS SEEDS FROM AMERICAN COAL FIELDS

FREDDA D. REED

(WITH TWENTY-SEVEN FIGURES)

Introduction

The early accounts of seeds or seedlike fructifications from the Paleozoic rocks of America, while made chiefly from casts and impressions, nevertheless demonstrate wide variation in form and size and consequently indicate a multiplicity of species. DAWSON, in his geological studies in Acadia (8, 9, 10, 11, 12), recognized sixteen species distributed in four genera. Although his descriptions are valuable from a taxonomic point of view, yet, as shown by STOPES (29) and from a reinvestigation of *Trigonocarpon hookeri* reported here, his interpretations—particularly of any cellular structure found therein—are often unreliable and even erroneous.

The reports of LESQUEREUX (18, 19), NEWBERRY (20, 21, 22, 23), WHITE (30, 31, 32), NOÉ (24), ARNOLD (2), DEEVERS (13), and others (1, 5, 14) greatly increased the number of species and added new terrains, until at the present more than 170 seed species have been figured, collected from the Upper Devonian and Carboniferous strata of North America, and geographically from Nova Scotia through the coal fields of eastern United States and west to Arkansas.

More recently the researches of KRICK (17), GRAHAM (15, 16), ARNOLD and STEIDTMANN (3), and DEEVERS (13), on the anatomy of seeds found in coal balls have not only contributed to our knowledge of their structure but have shown that there is variation in histological detail, thereby justifying in part the numerous species formerly reported. Owing to imperfection of fossilization and the age of the ovule when detached from the parent plant, superficial characters of form and size become of equivocal value in establishing new genera and even new species; but when variation in these characters is supported by variation in anatomical detail, then the existence of a multiplicity of species must be admitted.

Despite the number of seeds that have been investigated, no completely preserved specimens have been found in carboniferous strata; so incomplete are they that their exact nature is not known, as indicated by the fact that they have been variously referred to as fruits, nuts, ovules, seedlike, and seeds (17). Since information concerning their morphology becomes cumulative, each and every specimen retaining any cellular detail assumes an importance in helping eke out the story of their structure and evolution.

Observations

Conostoma oblongum Will.

The genus *Conostoma* was founded by WILLIAMSON (34) in 1877 to include three small seeds of the order Lagenostomales. The seed specified *C. oblongum* was found in the Lancashire coal fields, which belong in the Lower Coal Measures. In 1911 OLIVER and SALISBURY (27) published a detailed account of *C. oblongum* based upon an assemblage of all the known seeds of the species collected subsequent to WILLIAMSON's description. The comparatively few specimens of the assemblage, sixteen in all, led OLIVER and SALISBURY to state: "*Conostoma oblongum* is one of the rarest of the Paleozoic seeds." In 1932 KRICK (17) described briefly some specimens found in coal ball 213 of the Harrisburg, Illinois, collection. The incomplete preservation of her material, however, together with the obliquity of the preparations, made the seeds of little more value than to serve the purpose of identification and to report a new geographic origin and geologic stratum for the genus.

In addition to *C. oblongum*, other species of the genus have been described. Two of the three species upon which WILLIAMSON founded the genus, *C. ovale* and *C. intermedium*, were later reinvestigated by BENSON (4), who transferred them to another genus, *Sphaerostoma*, and reduced them to one species, *ovale*. OLIVER and SALISBURY established a second species, *C. anglo-germanicum*, for the reception of some specimens from Shore, Littleborough, England and from Duisburg in Rheinpreussen, Germany. GRAHAM (15) determined two more species, *C. platyspermum* and *C. quadratum*, found in coal balls from the Calhoun mine, Richland County, Illinois. The coal balls from this mine are of the same geologic age as

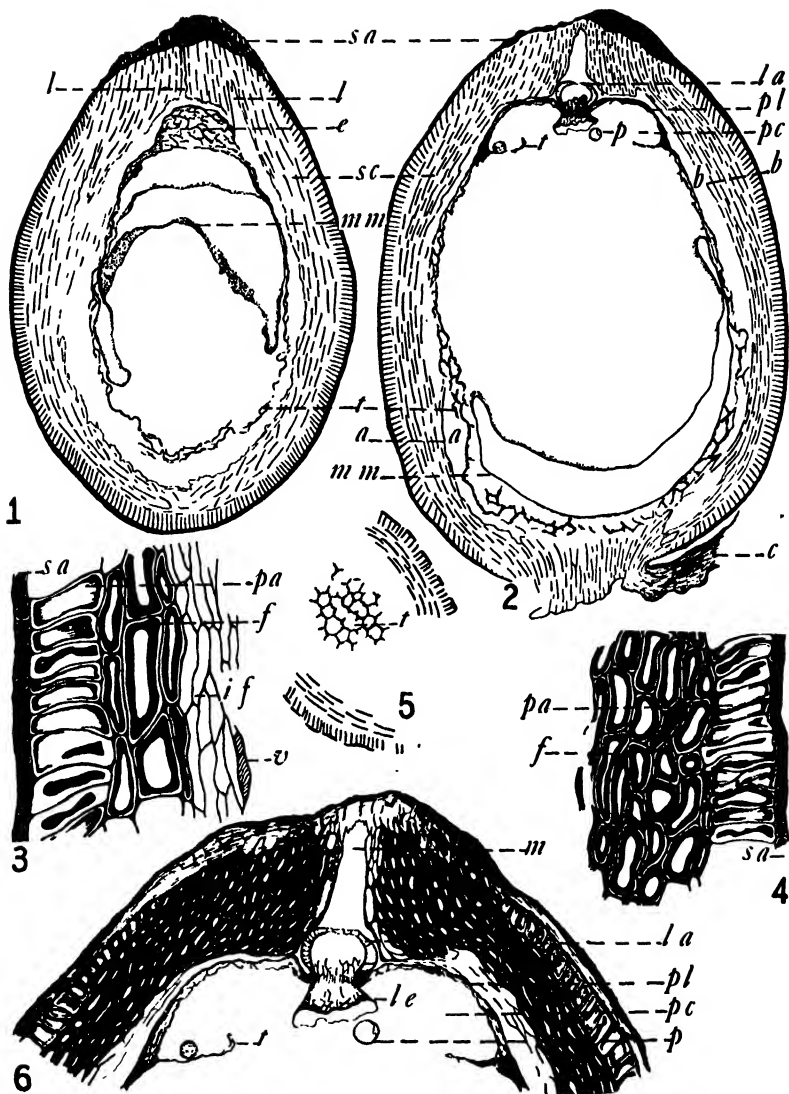
those from Harrisburg, the Allegheny group of the Upper Pennsylvanian. It is interesting to note that the British and European specimens have all been found in the Lower Coal Measures, whereas the American specimens of *Conostoma* have thus far been found in the Upper Coal Measures.

MATERIAL AND METHODS.—The following description is based upon four specimens found in coal ball 210 of the Harrisburg, Illinois, collection. Because of the small size of *C. oblongum*, the usual methods—the preparation of thin sections by cutting and grinding or the making of celloidin peels each with its consequent loss of material—were not applicable. Instead, sections of the coal ball, 1 mm. more or less thick, containing the seeds were ground with fine carborundum powder which served a double purpose, that of slowly abrading as well as maintaining a polished surface. At frequent intervals and as often as desirable the polished surface was coated with a thin film of Canada balsam, then examined under the microscope by means of reflected light and camera lucida drawings made. In such manner the specimen can be studied serially, and such small objects as the pollen grains, which measure about $80\ \mu$ in greatest diameter, can be observed in successive planes, making it possible to discriminate superficial markings from internal structure. Moreover, if at any time during the procedure a particularly desirable plane is revealed, that surface can be mounted permanently and the grinding proceed from the reverse side until the section is thin enough to be studied with transmitted light.

DESCRIPTION.—*Conostoma oblongum* is an elongate cylindrical seed about 4 mm. in length and 2.5 mm. in width. Of transverse sections only oblique ones were available, but these showed no evidence of platyspermy nor of ribs.

The seed remains consist of the integument, nucellus, and megaspore membrane. Within the pollen chamber are some pollen grains and at the base of one of the specimens there was some adherent tissue which appeared to be a remaining fragment of the cupule (fig. 2c). Save for the one remaining fragment there was no vegetative tissue in the coal ball in organic connection with the seeds.

The integument, which around the body of the seed is about 0.3 mm. thick, consists of three distinct zones termed by OLIVER and



FIGS. 1-6. —*Conostoma oblongum*. Fig. 1, tangential longisection: *sa*, sarcotesta; *l*, loculus containing vascular tissue shown in fig. 8; *e*, epidermis of nucellus; *sc*, sclerotesta; *mm*, megaspore membrane; *t*, tapetal layer of nucellus. \times about 20. Fig. 2, nearly median longisection: *la*, lagenostome; *pl*, plinth; *pc*, pollen chamber; *p*, pollen; *c*, fragment of cupule; other lettering as in fig. 1. \times about 20. Fig. 3, detail of portion of integument from *a-a* of fig. 2: *pa*, palisade layer of sclerotesta; *f*, fibrous layer of same; *if*, inner fleshy layer of integument showing traces of vascular tissue, *v*. \times about 95. Fig. 4, detail of integument from *b-b* of fig. 2. \times about 95. Fig. 5, tangential longisection showing surface view of tapetal cells, *t*. \times about 16. Fig. 6, detail from micropylar end of fig. 2: *m*, micropyle; *le*, lenticular nucellar tissue. \times about 40.

SALISBURY the sarcotesta, sclerotesta, and endotesta or inner fleshy layer.

The sarcotesta is the investing layer and consists of epidermis only. In this material it is poorly defined, both by reason of the dense contents which obscure the limitation of the cell walls and because of the incomplete preservation. Around the body of the seed the epidermis appears to be fairly uniform; in longitudinal section the cells are square to elongate and about $25\ \mu$ thick. At the micropylar region the epidermal cells become radially elongated, thereby increasing the thickness of the layer to more than $100\ \mu$ (fig. 1).

Underneath the epidermis is the sclerotesta, composed of two types of cells forming two well differentiated layers. The outer layer is of radially elongated, palisade-like cells, $30\ \mu$ wide by $120\ \mu$ in radial depth (figs. 3, 4). These cells are likewise filled with some organic material, but it is not so dense and opaque as that contained in the epidermis, and instead of appearing black like the epidermis these cells are golden brown. The uniseriate palisade-like cells inclose the inner layer of the sclerotesta, the cells of which are differentiated by being vertically elongated, fibrous, and multiseriate, of three to seven rows (figs. 3, 4). At the micropylar region these cells increase in number, resulting in a perceptible increase in the thickness of the sclerotesta (fig. 6).

Of the three zones of the integument, the inner one shows the least preservation, doubtless because of the structure of the cells, which are thin-walled, parenchymatous, and vertically elongate. Occasional fragments of this tissue are found in which tracheids with spiral to scalariform thickenings are seen. The inner cells of this zone are always in a fragmented condition (fig. 3).

Because of incomplete preservation the vascular system is not intact, yet the remaining fragments of conducting elements are indicative of some facts with regard to its course. From the chalazal end discrete strands pass up through the inner fleshy layer almost to the level of the summit of the nucellus, and then, accompanied by thin-walled parenchyma, they penetrate the fibrous layer of the sclerotesta (figs. 1, 7, 8) in which they terminate. The number of bundles, determined by the loculi in the distal end of the sclerotesta (fig. 1),

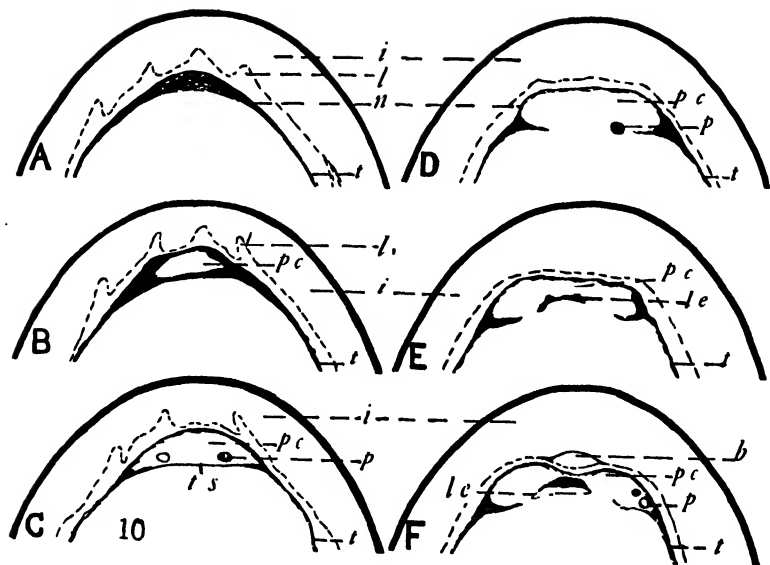
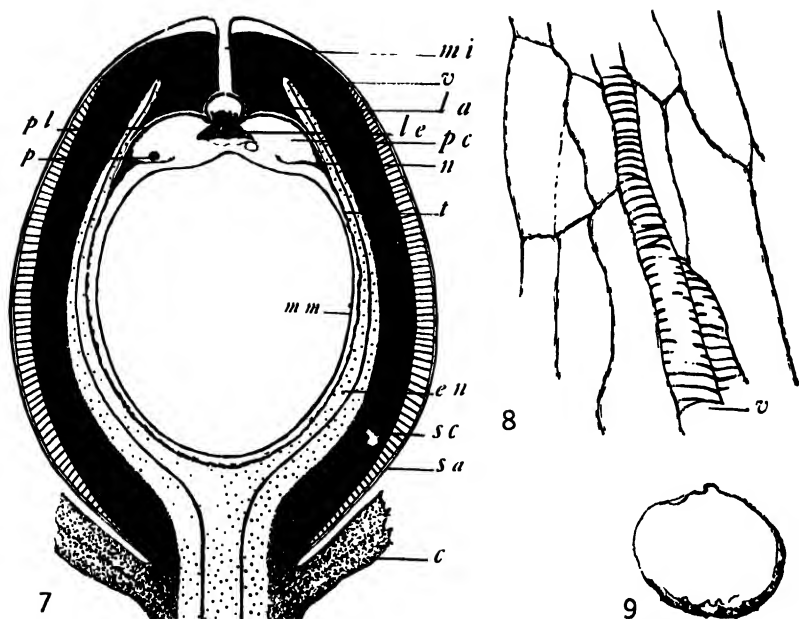
which were formed by disorganization of the soft tissues accompanying the vascular elements, was at least six and probably eight.

The nucellus and integument appear to be nondiverged to the floor of the pollen chamber. Owing to the lack of preservation, the exact point of divergence of the two structures is another moot question.

In their description of *Conostoma*, OLIVER and SALISBURY defined three regions of the nucellus: (1) the main body of the nucellus or megaspore chamber, surmounted by (2) a truncated continuation, the plinth, at the summit of which is a depression in which is (3) the lagenostome. Of the three regions of the nucellus in the specimens under consideration there is little remaining save the limiting layers, which are the epidermis and tapetum (figs. 5, 6). From the point of divergence of the integument and nucellus, the epidermal layer follows the contour of the inner layer of the integument to the base of the micropyle, where it turns in and down, making a depression at the apex of the plinth region. Then, leaving a small orifice about $135\ \mu$ in diameter, it expands to form a globular body which fits into the apical depression of the plinth and under the beveled edges of the integument at the base of the micropylar tube (fig. 6). These last epidermal cells are the limiting cells of the lagenostome; while they are continuous with the epidermal cells of the plinth region, yet they differ by the possession of delicate scalariform sculpturing on their walls (fig. 6).

The lagenostome is hollow, and measures about $130\ \mu$ deep by $180\ \mu$ wide. At the apex there is a small orifice directly under and opening into the micropyle. The lenticular mass of tissue (fig. 6le) is not interpreted as being an integral part of the lagenostome but intruded tissue of the plinth, and will be considered later.

The plinth is bounded peripherally by its epidermis, distally is surmounted by the lagenostome, and proximally is separated from the megagametophyte by the tapetal septum. It is about 1 mm. in diameter at the base and about 0.25 mm. deep. The epidermal cells are large and flat and have thicker walls than those of the tapetum. Figure 10 shows a series of somewhat oblique longitudinal sections through the distal end of the seed of successive planes of the plinth from tangential to almost median aspect. In A the epidermis of the



FIGS. 7-10.-*Conostoma oblongum*. Fig. 7, reconstruction of median longisection. $\times 30$. Fig. 8, vascular elements from loculus of fig. 1. \times about 500. Fig. 9, section of pollen grain found in pollen chamber. \times about 500. Fig. 10, series of longisections through micropylar end of seed, showing successive planes of nucellus. $\times 30$.

plinth is seen in surface view; *B* is through the peripheral region of the plinth and pollen chamber; in the plane of *C* the tapetum appears as an unbroken septum over the megaspore membrane and two pollen grains are seen in the pollen chamber; at *D* there is a break in the tapetal septum, which in the next plane, *E*, is capped with tissue that seems to be a single layer of cells of the same texture as those of the tapetum. The progressively more median sections (fig. 10*F*), and figure 6, show this tissue as a lenticular layer with the convex side extending into and plugging the lagenostome. In these specimens of *Conostoma*, therefore, the lenticular mass of tissue, which in the former descriptions (27, 15) is of conjectural history, is here interpreted as the remains of the tapetal layer. By reason of the thicker walls of its cells it did not deliquesce as did the internal tissue of the plinth in the formation of the pollen chamber, and after pollination was forced up through the plinth cavity into that of the lagenostome by a tent-pole development of the megagametophyte. In such manner the pollen grains would be effectively sealed in the pollen chamber.

Numerous pollen grains were found in the pollen chamber (figs. 2, 6). In one specimen ten were counted, and if they were uniformly distributed throughout the pollen chamber at least a third more must have been lost by the cut of the saw. The pollen grains are ellipsoidal, about $50\ \mu$ wide by $80\ \mu$ long. They have superficial reticulated markings and sometimes the walls appear to be wrinkled or slightly folded, but when seen in optical section they show no internal structure nor any indication of being multicellular (fig. 9). A unicellular condition of pollen grains would be at variance with the previous reports for *Conostoma*. Likewise, by analogy with modern gymnosperms, it would be contrary to the condition one would expect to find at this late stage in the ontogeny of the seed. Failure to find cells within the pollen grain might be attributed to lack of preservation, or it may be that the pollen grains are simply the exine remains after the formation and escape of spermatogenous cells.

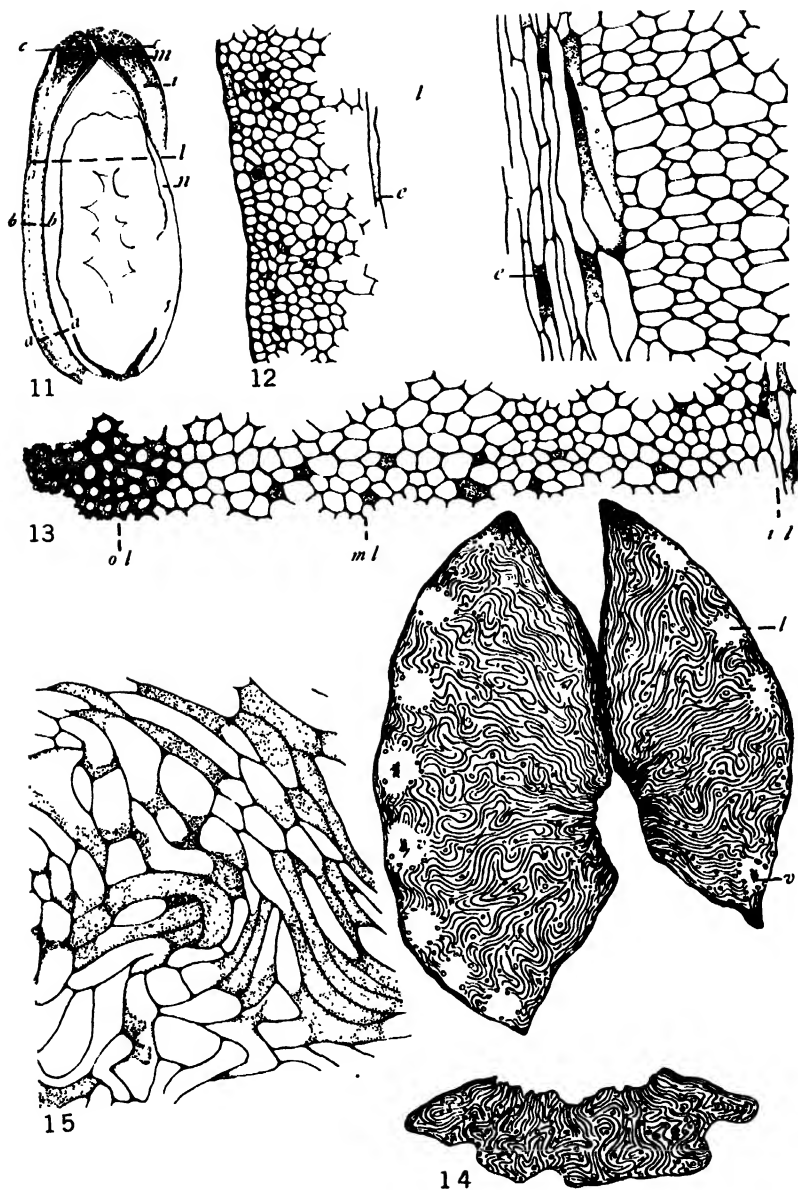
Pachytesta gigantea Ad. Br.

Two specimens of *Pachytesta*, referred to here as *Pachytesta* no. 1 and no. 2 respectively, were found by No   at Polk's Patch near

Brownsville, Indiana. The coal here is also no. 5 of the Allegheny group of the Upper Pennsylvanian.

PACHYTESTA NO. 1.—This specimen has been figured under the name *Trigonocarpus* (7), which term was based upon the superficial character of the exposed portion of the seed and applied before its internal structure was known. When the coal ball containing the seed was sectioned, however, the previously labeled "seed" was revealed as a cast of the inner lining of the integument. It was also clear that the three ridges, which were largely responsible for the term *Trigonocarpus*, were not ribs of the integument as formerly thought, but mark the sutures or dehiscence slits of the integument. Since the integument has disappeared from the chalazal end the size of the seed can only be approximated, but a conservative estimate would make it about 7 cm. long by 3 cm. in transverse diameter. Exclusive of the integument, it measures 5.5 cm. long by 2 cm. in diameter.

A median longitudinal section through the chalazal end (fig. 11) indicates the relationship of the tissues in so far as they are preserved. The integument is thick and compact, hence the term *Pachytesta*. A detail through the integument shows three poorly defined layers (fig. 13). The outer layer, composed of isodiametric, relatively small, thick-walled cells, merges imperceptibly into the middle layer, the cells of which are distinguished from those of the former only by their slightly larger size and thinner walls. The middle layer in turn gives way to the inner layer, which is made up of several rows of thick-walled, elongated cells. While these elongated cells are the innermost ones remaining, yet it is not believed that they represent all the inner layer, but rather that they may have bordered thin-walled tissue, corresponding to the inner fleshy layer of modern gymnosperm seeds, which was not preserved probably because of the soft texture of the cell walls. At the micropylar end the integument is thicker, owing more to the character of the cells than to an increase in their number, for here they are larger in caliber, elongated, and sinuous (fig. 15). A transverse section at this level (fig. 14) shows the three valves of the integument, in the peripheral region of which are clear areas left by the disintegration of tissue. In some of these loculi are traces of organic material which



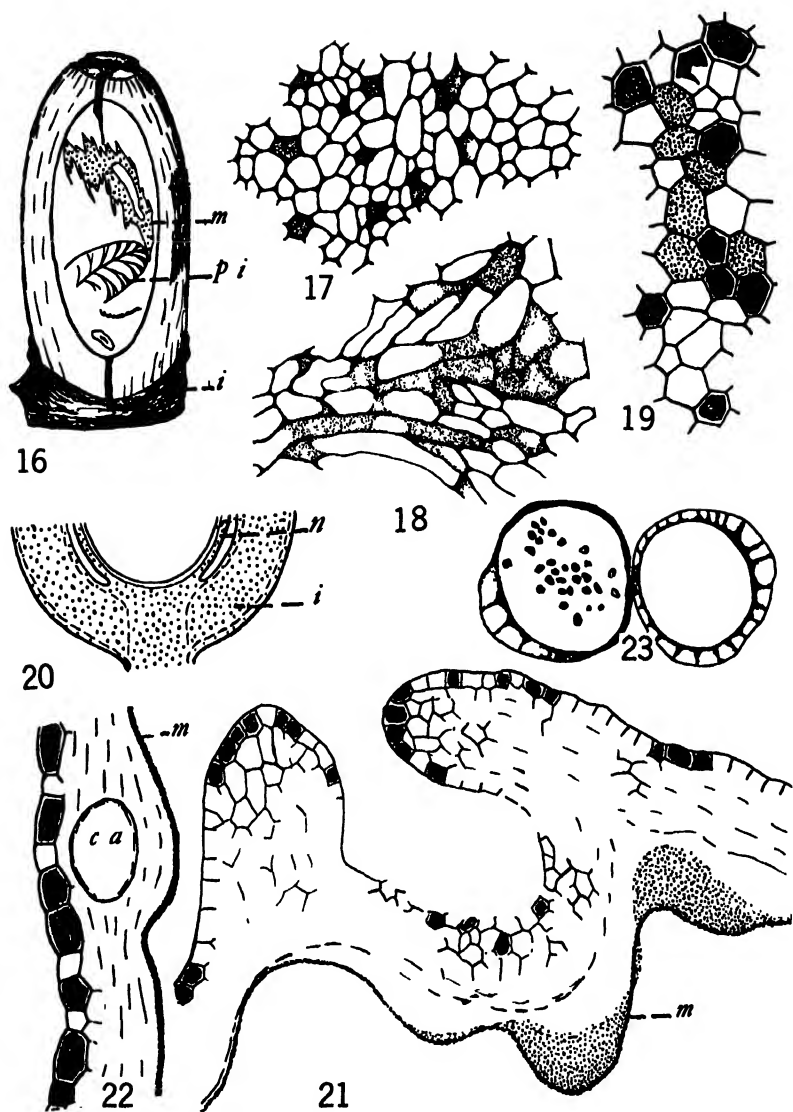
FIGS. 11-15.—*Pachystea gigantea* no. 1. Fig. 11, diagrammatic representation of median longisection: *m*, micropyle; *i*, integument; *l*, loculus with indications of vascular tissue; *n*, nucellus. \times about 1. Fig. 12, portion of longisection of integument at level of *b-b* of fig. 11, showing elongated cells, *e*, which border the loculus. \times about 40. Fig. 13, portion of longisection of integument at level *a-a* of fig. 11 showing: *ol*, outer layer; *ml*, middle layer; *il*, inner layer. \times about 40. Fig. 14, diagrammatic representation of transection of integument at level *c-c*: *v*, probably remains of vascular tissue. Fig. 15, detail of few cells of fig. 14. \times about 40.

may well be remains of conducting elements, since it was from this region that BRONGNIART (6) and OLIVER (25) found and figured vascular strands. In longitudinal section these loculi are seen bordered by elongated cells which in texture and appearance simulate the elongated cells of the inner layer of the integument (figs. 12, 13).

In most of the seeds that have been described of the order Trigono-carpaes (the seed order in which *Pachytesta* belongs) two vascular systems are recorded: an outer one which diverges from the main supply of the ovule at the chalaza and traverses the outer layer of the integument, and an inner system which is an extension of the chalazal bundles penetrating the nucellus to the floor of the pollen chamber. This latter system is described as consisting of a tracheal mantle as in *Stephanospermum* (26), or as being composed of discrete bundles as in *Trigonocarpus*. Since there were no recognizable vascular elements in this specimen of *Pachytesta*, it is hazardous to describe their possible position. Nevertheless, if the elongated cells of *il* figure 13 and *e* figure 12 are regarded as being of the nature of a bundle sheath, then the evidence indicates a double system confined to the integument: an outer system of twenty or more strands penetrating the outer layer of the integument and represented by the loculi (*l*) of figures 11 and 14 and an inner system of fewer strands all traces of which have disappeared except for the bordering elongated cells preserved as the inner limit of the integument.

The nucellus and adherent megaspore membrane are indistinct. This is not altogether because of poor preservation, for the nucellus has a shriveled appearance as if it had reached a membranaceous state before petrification occurred, as happens late in the ontogeny of modern gymnosperm seeds. It is therefore of some value in indicating an advanced stage in the development of the ovule, but the limited amount of preservation precludes any additional information in the nature of a contribution to the anatomy of the seed. There was no tissue preserved within the megaspore membrane.

PACHYTESTA NO. 2.—The integument had almost disappeared from this specimen except for some fragments near the chalazal end (fig. 16), but of these remaining fragments there was enough de-



FIGS. 16-23.—*Pachylesta gigantea* no. 2. Fig. 16, seed shown diagrammatically after removal of first section: *m*, megaspore membrane; *pi*, one of intruded pinnules; *i*, integument. About natural size. Figs. 17, 18, details of cellular structure of integument. \times about 40. Fig. 19, surface view of epidermis of nucellus. \times about 40. Fig. 20, diagram from median longitudinal section of chalazal region showing divergence of integument, *i*, and nucellus, *n*. Figs. 21, 22, sections through nucellus and megaspore membrane. \times about 35. Fig. 23, two of sporangia, one containing spores, found in region of megagametophyte. \times about 70.

tail of structure to establish relationship with the former specimen. This seed is slightly smaller than *Pachytesta* no. 1; exclusive of the integument it measures 4.8 cm. long by 2.2 cm. in transverse diameter.

The seed was cut at right angles to the plane of one of the ribs. Also median sections were secured through the chalazal and micropylar ends. Removal of the first section exposed the surface shown in figure 16. This surface would furnish almost a complete description of the seed as preserved, for the succeeding cuts supplied little additional information aside from making it possible to trace the course of the nucellus. Yet there are certain details of the tissues that are worth recording.

In the *Trigonocarpaceae* the nucellus and integument diverge in the chalazal end of the ovule (fig. 20). The nucellus consists of several layers of cells, the exact number not determined, of which the outer layer or epidermis is very conspicuous. A surface view of a group of these cells shows them to be roughly isodiametric, relatively thin-walled, and many of them filled with dark colored material (figs. 19, 21, 22). This last feature gives the layer so characteristic an appearance that the epidermis can be identified however fragmentary or disarranged it may be. Within the epidermis the amount of preservation is scanty. Only rarely is it possible to get the outline of a cell, but the few remaining ones do not appear to have collapsed and become membranaceous as in the former specimen. Such cavities as the one at *ca*, figure 22, are found in this tissue. They seem too regular to be the result of some chance irregularity of fossilization, and have the appearance of mucilage ducts. The preservation is so meager, however, that their nature is purely speculative. There is no trace of nucellar vascular tissue.

Within the nucellus and more or less adhering to it is the megaspore membrane, notable for its thickness. In modern seed plants the thickest megaspore membrane is found in the most primitive order, the *Cycadales*, where it may measure up to 10 μ thick, depending on the species and the stage of development. In *Pachytesta* the megaspore membrane attains a thickness of 15 to 20 μ . This exceedingly thick membrane might have been postulated for a group of plants at the level of the *Cycadofilicales*, the order of gymnosperms in which

Pachytesta belongs, since they must have evolved from a more primitive heterosporous condition in which the megaspores were shed earlier in their ontogeny and would need a thick membrane functioning as a protective layer.

These two layers, the nucellus and adherent megaspore membrane, are not found in their normal position following the contour of the integument; on the contrary they are much disarranged (fig. 16). This condition is not thought to have resulted from disorganization of tissues caused by germination of the embryo, rather it seems to be the result of some mechanical injury during development of the megagametophyte while the tissues were soft and pliable. The condition of the few remaining nucellar cells indicates that they were in a state of functional activity at the time petrification occurred. That there was some mechanical injury is further evidenced by the intruded rootlet, pinnules, sporangia, and other fragments of plant tissues (fig. 16). The absence of gametophyte or of embryonic tissue within the megaspore membrane would not therefore be accounted for by the germination and escape of the embryo.

Although proximity of plant structures, without actual organic connection, is no basis for establishing relationship, yet it is interesting and significant that the intruded pinnules, one of which is outlined in figure 16, have the recurved margins, the type of venation, and the structure of *Alethopteris*, a leaflet ascribed to *Medullosa*, the stem genus to which *Pachytesta* has been assigned.

Discussion

The foregoing descriptions but add to the mounting list of Paleozoic seeds or seed remains in which no embryonic tissues have been found. Theories, almost as numerous as the number of investigators, have been formulated for explaining the absence of embryos. None of them seem tenable, however, inasmuch as they presuppose certain given conditions; and while the given conditions may have existed and been responsible for the degree of preservation of the seed remains which suggested them, yet it would seem that these conditions or combination of conditions are fortuitous, and that it is possible and probable that Paleozoic seeds with embryos may be

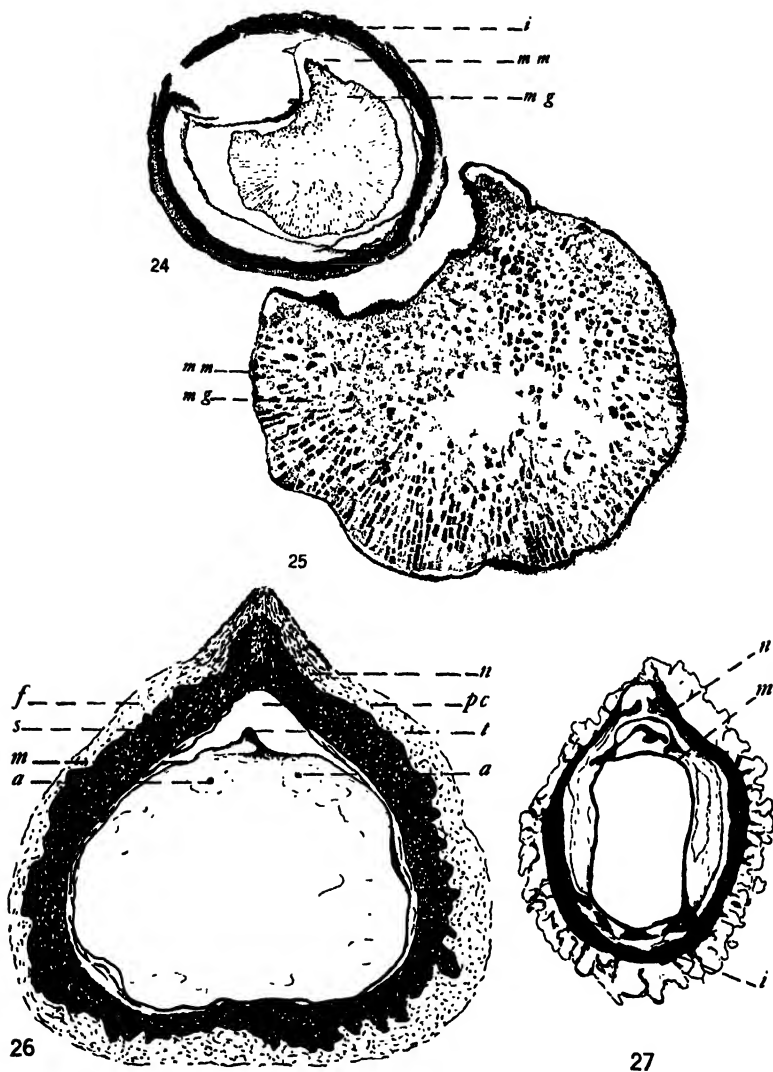
found.¹ That Paleozoic seeds are not peculiar or very different from their modern descendants in their method of development, and that there was variation in their age at the time of fossilization, is illustrated in the following series:

The transverse section of *Lagenostoma ovoides* (figs. 24, 25) has the resulting radial arrangement of cells of the endosperm or megagametophyte when the procedure of development is free nuclear division, partial placement, and subsequent centripetal wall formation. Without the median longitudinal section of this ovule one can only speculate on the reason for the sparsity of cells in the center. It may be that the process of wall formation had not progressed to completion; that wall formation ceased before segmentation was complete, as sometimes occurs in the ovules of modern gymnosperms; or that this tissue, lying in the path of an advancing embryo, was broken down and resorbed by it. This last condition could likewise find its counterpart in modern gymnosperms. It is to be regretted that there are no serial sections of this ovule available, for with such remarkable preservation, either archegonia or young embryos would surely be present.

While there is no endosperm preserved in *Pachylesta* no. 2, the condition of the nucellus suggests that the endosperm was in a developmental stage and had probably advanced to the formation of archegonia at the time petrification occurred.

The sharply differentiated and thick-walled tissues of the integument of *Conostoma oblongum* are characteristically mature. The presence of pollen grains in the nucellus not only establishes the incident of pollination, but their being devoid of contents indicates an ensuing period of development of microgametophyte. That there was a developmental period of the microgametophyte is supported by the condition of the nucellus, which, being the source of nutrition for the further development of the pollen grains after pollination, has here lost all of its internal tissue in the micropylar region as it would had it been absorbed by the functioning microspores. The proportionate size of the endosperm, as represented by its limiting layer the megaspore membrane, and the tent pole development indicated in

¹ Since the above was written, DARRAH has announced the discovery of *Cardiocarpon* embryos. Amer. Jour. Bot. 25:98. 1938.



FIGS. 24-27. —*Lagenostoma ovoides*. Fig. 24, diagram of transection: *i*, integument; *mm*, megaspore membrane; *mg*, megagametophyte. \times about 20. Fig. 25, detail of megagametophyte of fig. 24. \times about 45. This section of *L. ovoides* (from British Coal Measures) kindly lent by Dr. G. R. WIELAND of Yale University, who has previously figured it (33). Fig. 26, *Cardiocarpon*, semidiagrammatic longisection: *f*, outer fleshy layer of integument; *s*, stony layer; *n*, nucellus; *pc*, pollen chamber; *t*, tent-pole development of megagametophyte; *m*, megaspore membrane; *a*, organic material occupying position of nuclei of fertilized eggs. \times about 6.5. Fig. 27, *Trigonocarpon hookeri*, tangential longisection showing: *n*, nucellus; *m*, megaspore membrane; *i*, integument. This specimen, figured by DAWSON (12), lent by Redpath Museum of McGill University.

this material and demonstrated in the British specimens, are again evidence of a late stage in the history of the ovule. Yet the undisturbed appearance of the micropylar region of the nucellus, particularly the very small openings at both the base and apex of the lagenostome, makes it impossible to reconcile these specimens with "seed remains" after the germination and escape of the embryo.

Another seed significant in the developmental chain is that of *Cardiocarpon* (28). The preservation does not warrant a conclusive statement concerning the organic material at *a*, figure 26, but the position of these two bodies seems too regular to be chance. In its tent-pole prolongation and in its broad outline, the endosperm of *Cardiocarpon* presents a striking resemblance to that of *Ginkgo*. If one assumes a parallel development of the endosperm of the two genera, then the two dense bodies occupy the position of fertilized eggs. Were they the egg nuclei one would expect to find them toward the micropylar end of the central cell rather than centrally located as these appear to be.

The larger size of *Pachytesta* no. 1 and the membranaceous character of the nucellus indicate that this specimen may have proceeded to an embryonal stage; however, as in *Conostoma*, the integument and nucellus do not appear to have been disturbed by the emergence of a germinating embryo.

Any later stages of embryogeny that have been recorded are even more doubtful. DAWSON (12) writes of embryos and has one figured,—here reproduced in figure 27. The type specimen from which his drawing was made, together with some other seeds of the same collection, was kindly lent by the Redpath Museum of McGill University, with the permission to reinvestigate them. Figure 27 is labeled according to my interpretation. There seems no doubt that *m* is the megaspore membrane, and no doubt that there is no organic tissue within it. An examination of some of the other seeds of the collection showed them to be of similar preservation.

Summary

1. Structurally preserved seeds were found in coal ball material collected under the auspices of the Illinois State Geological Survey by Dr. A. C. NOÉ of the University of Chicago. The source of the

coal balls was from southern Illinois and Indiana in coal no. 5, which is in the Allegheny group of the Upper Pennsylvanian.

2. The seeds described are *Conostoma oblongum* Will. of the order Lagenostomales, and *Pachylesta gigantea* Br. of the order Trigonocarpaceae. This is the first description of *Pachylesta* from American coal balls.

3. Illustrations of *Lagenostoma ovoides* from the British Coal Measures, and of *Pachylesta gigantea*, *Conostoma oblongum*, *Cardiocarpon*, and *Trigonocarpon hookeri* from the American Coal Measures, are used to demonstrate similarity in development of Paleozoic seeds with those of modern gymnosperms, and to show variation in ontogeny at the time of petrification.

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TRANSPLANTATION EXPERIMENTS WITH PEAS. II

H. E. HAYWARD AND F. W. WENT

(WITH FOUR FIGURES)

Introduction

Previous experiments on the grafting of pea seedlings had led to the conclusion that: "Until actual union of the grafted peas through junction of the tissues by regenerated vascular bundles had occurred, no growth of the grafted tips, either of stems or leaves, took place" (4). Owing to the fact that many special cases were encountered in the grafting experiments which did not fit this conclusion, an anatomical study was made of the graft union to determine whether the type and rate of growth of scion were correlated with the anatomy of stock and scion, particularly with reference to the approximation of the original vascular tissues and the differentiation of new vascular connections. In addition, a number of grafting experiments were carried out to check certain details which had arisen as a result of the anatomical investigation.

The technique employed was that used by WENT (4). The axis of an etiolated seedling was cut obliquely through the median portion of the first internode, and the second internode was removed by making a similar cut through the third internode.¹ The two cut surfaces were then fitted together and held in place by a piece of glass tubing 5 mm. long, with a bore about equal to the diameter of the seedling axis. In early experiments, lack of good contact between the opposed surfaces of the graft was sometimes responsible for the failure of the graft to "take." The use of a device resembling a miniature miter-box to guide the razor blade eliminated this difficulty to a considerable degree, as the ends of the stock and scion could be cut at the same angle.

Alaska peas were germinated and grown in the physiological dark-room for one week; the graft was then prepared and daily length measurements of the entire axis were taken over a period of several

¹ The writers wish to express their thanks to Mr. G. C. WARNER for his assistance in the grafting experiments.

days. Seedlings representing each of the growth types were selected, the portions of the axes which included the graft were excised, killed, imbedded in paraffin, and serial longitudinal sections cut at 10 μ .

Investigation

ANATOMY OF FIRST AND THIRD INTERNODES

The structure of the lower internodes of the pea has been described by HAYWARD (1). Owing to the peculiar character of the transition zone, which includes the first three internodes, it is possible to distinguish between the first and third internodes in serial longisections. It is rather simple, therefore, not only to analyze the degree of vascular approximation of the original bundles and the amount of effective vascular tissue differentiated, but to determine the relative activity of the stock and scion.

In the first internode the vascular tissues of the stele consist of six groups of primary phloem, each of which is bounded on its outer face by a strand of phloem fibers and two distinct regions of primary xylem (fig. 1A). As seen in transection, the centrally located xylem consists of two crescentic strands whose convex surfaces are usually in contact at the center of the axis. There is some variation in this regard, and at the upper limits of the internode there may be a narrow band of parenchyma separating the two metaxylem groups. The four groups of protoxylem which form the peripheral portions of the central xylem strands are directed toward the two polar bundles of the stele, so that the relation of the protoxylem and metaxylem is exarch. The polar bundles which comprise the second region of the stele are endarch and collateral. There is a single-layered pericycle which is limited centrifugally by an endodermis with well defined Casparian thickenings. Cambium develops in the two polar bundles and in the zone of fundamental parenchyma between the centrally located metaxylem and the laterally placed phloem groups. This is relatively more active than the cambium of the root and may play a part in the formation of new tissues at the graft union (fig. 3A).

In the third internode the primary phloem groups and the polar bundles are oriented much like those of the first, but the laterally

oriented bands of xylem are widely separated by a central pith. The protoxylem and metaxylem are tangentially mesarch, with the protoxylem centrally located on the inner face of each band and flanked by groups of metaxylem elements (fig. 1*B*).

The cortical tissues are organized in an identical manner in the two internodes. There are two fiber bundles that lie in the same vertical plane as the polar bundles of the stele, and two fibrovascular

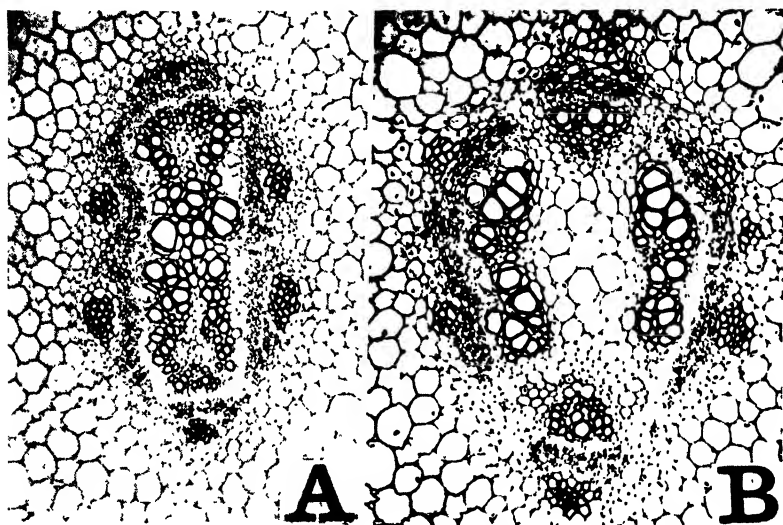


FIG. 1.—*A*, transection through stelar portion of first internode, showing arrangement of vascular tissues. *B*, same for third internode.

bundles that lie at right angles to them. In some cases large lacunae may develop in the parenchyma between the cortical bundles, but this was not observed in the young axes investigated in these experiments.

HISTOLOGY

The protoxylem consists of a limited number of slender annular and spiral elements which were probably nonfunctional at the time the graft was made. They appear much stretched as a result of axial elongation, the primary walls are collapsed, and in some cases the annular and spiral secondary wall thickenings are canted or pulled lengthwise. The vessel segments of the metaxylem are

reticulated or pitted, three to five times as long as broad, with transverse end walls. When present, the secondary xylem consists

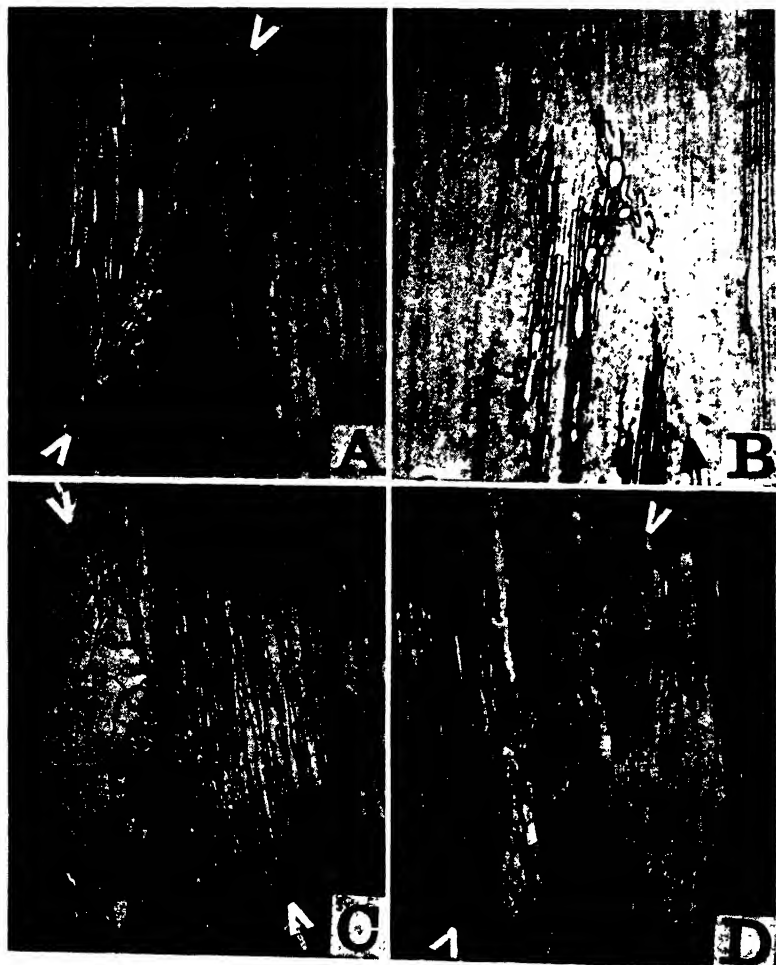


FIG. 2.—Longisections through graft unions. *A*, poor approximation of vascular elements of stock and scion and differentiation of new vascular tissue. *B*, same but with differentiation of new elements in scion. *C*, fair approximation of vascular elements with new xylem elements differentiating at junction of union. *D*, fair approximation of xylem elements and relatively good apposition of phloem. Scions are uppermost; arrows indicate plane of union.

of pitted vessels and xylem parenchyma. The vessel segments have greater diameter and less length than those of the metaxylem. In

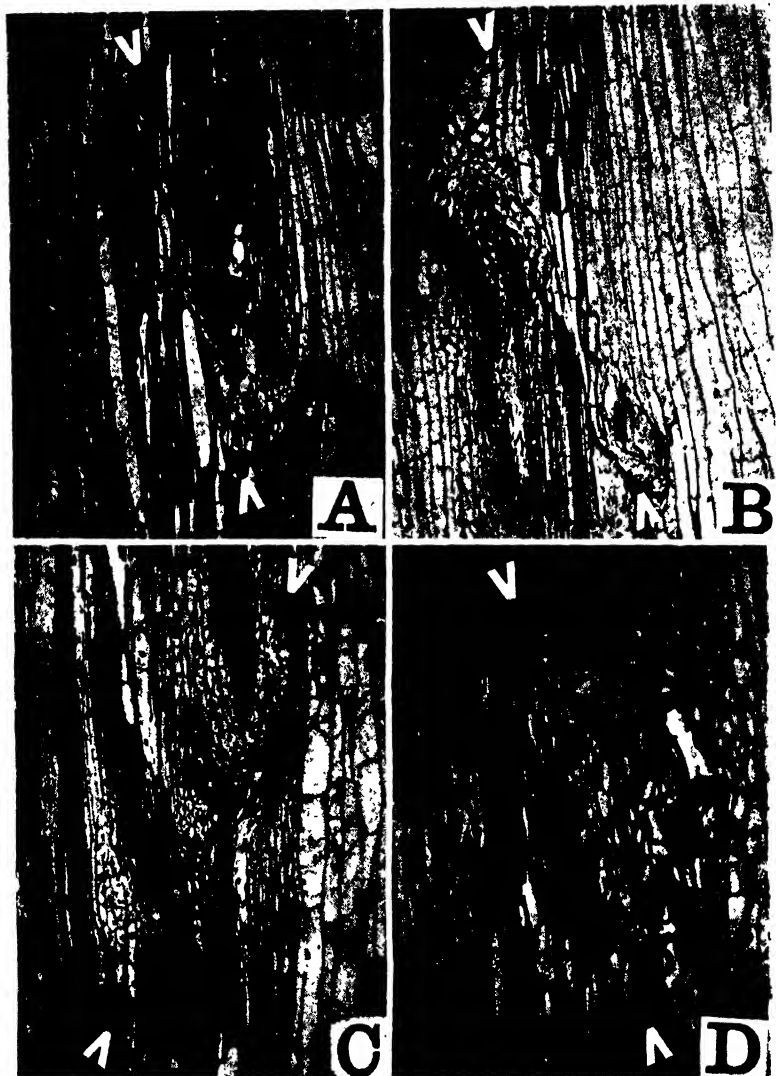


FIG. 3.—Longisections through graft unions. *A*, good approximation of xylem elements and accompanying cambial activity. *B*, fair approximation of xylem elements, formation of wound tracheids, and accumulation of deep-staining substances in vessels of stock. *C*, *D*, formation of new connecting vascular strands in cases where original approximation was fair (*C*) and poor (*D*). Scions are uppermost; arrows indicate plane of union.

the larger vessels there was frequently an accumulation of deeply staining substances which apparently plug up the lumina of the vessel segments for some distance from their cut ends (figs. 2*D* and 3*B*).

The wound tracheids which are formed in the parenchyma adjacent to original vascular tissue are irregular in shape and size, with reticulate or pitted walls. They are roughly isodiametric, or two to three times as long as broad. Those formed from cambium are laid down in rows and are more regular in shape, resembling the secondary vessels in this respect (fig. 3*A*).

The primary phloem consists of long slender sieve tubes and companion cells with adjacent strands of much elongated, thick-walled phloem fibers. There is relatively little primary phloem parenchyma. The cells of the wound phloem are usually irregular in size and shape, and in many cases resemble parenchymatous cells.

There is a definite spatial relationship between the new vascular tissues and the original vascular elements. In regions where the cut ends of xylem and phloem elements abut parenchyma, the activity of the latter is much accelerated, and it is at such loci that the majority of the wound tracheids and phloem elements are differentiated (fig. 2*A*, *B*). Although there is activation of the parenchyma on both sides of the graft, in general the parenchyma of the stock is much more active than that of the scion. In twenty observed grafts, meristematic activity and the differentiation of new vascular elements was greater in the stock in fourteen instances, and approximately equal in stock and scion in three other cases (fig. 2).

ANATOMICAL ANALYSIS OF GRAFT TYPES

The growth in length of 180 grafted peas was followed daily; and five and nine days after grafting, groups of peas representative of four different types of response were selected for anatomical investigation. In this series of grafts, a large number of grafted shoots started to grow rapidly immediately after grafting. In most experiments of this type this phenomenon is very rare, but fortunately it could be investigated in this case. Groups I to IV were collected five days after grafting; groups V to VII, nine days after grafting. Table 1 gives the mean growth in millimeters per day for each group.

A brief description of the anatomy of the graft in each group follows. It should be noted that in all seven groups, formation of new vascular tissue occurs to some extent. The differentiation of these tissues is usually initiated in the parenchyma abutting the cut ends of the vascular elements of the stock or scion, and in the parenchymatous regions adjacent to the vascular strands in either segment of the graft (fig. 2). In some instances the pericyclic tissue may be active in the formation of vascular elements; in others there was evidence of cambial activity (fig. 3A). In some of the types to be described, effective continuity of the vascular tissues of the stock and scion was not established during the growth period observed.

TABLE 1
MEAN DAILY GROWTH INCREMENTS FOR EACH GROUP (MM.)

GROUP	TYPE	FIRST DAY	SECOND DAY	THIRD DAY	FOURTH DAY	FIFTH DAY	SIXTH DAY	SEVENTH DAY	EIGHTH DAY	TOTAL
I. . . .	D	2.7	0.2	1.2	0.7	14.0
II. . . .	B	4.2	4.0	1.2	1.2	10.7
III. . . .	C	9.0	12.3	7.3	6.0	34.6
IV. . . .	A	10.0	17.2	5.2	2.5	35.0
V. . . .	A	10.5	12.5	10.5	6.0	5.0	2.0	3.0	0.0	40.5
VI. . . .	D	4.0	0.5	1.5	3.0	3.0	3.5	19.0	20.5	55.0
VII. . . .	C	7.5	9.0	7.0	12.0	10.5	27.5	44.5	21.0	139.0

GROUP I.—Growth response was characterized by little or no axial elongation during the first three days, followed by abrupt initiation of growth. This occurred in 10 per cent of all cases. The approximation of the original vascular elements of stock and scion was uniformly poor, with no direct juncture at any point in some instances. There were, however, effective vascular connections resulting from the differentiation of new xylem and phloem elements (fig. 2B).

GROUP II.—Growth response was characterized by little or relatively slow axial elongation, with gradual reduction in the daily rate and later complete cessation. Growth in length proceeded at the rate of a few millimeters a day, but after three days it had stopped completely. This occurred in 14 per cent of all cases. There was rather poor approximation of the original vascular elements of stock

and scion, and slight or no differentiation of effective new vascular tissue connecting stock and scion.

GROUP III. --Growth response was characterized by rapid initial axial elongation which continued at a relatively even rate. This occurred in 8 per cent of all cases. There was good approximation of the original vascular elements of stock and scion, and fair to good differentiation of effective new vascular tissue (fig. 2C).

GROUP IV.—Growth response was characterized by rapid initial axial elongation followed by slowing down or cessation of growth. Increase in length proceeded rapidly for the first two or three days, with subsequent reduction in the daily rate. This occurred in 68 per cent of all cases. There was good approximation of the original vascular elements, but very poor or no development of effective new vascular connections between stock and scion.

GROUP V.—Growth response was characterized by rapid initial axial elongation followed by cessation in the last three days. The growth period in this group was three days longer than in group IV. As in group IV, there was fair to good approximation of the original vascular elements, with poor or at best fair development of effective new vascular connections.

GROUP VI.—Growth response was characterized by slow initial growth and then rapid axial elongation. There was poor to fair approximation of the original vascular tissues, with good development of effective new vascular connections. Well defined bundles were differentiated which established continuity between stock and scion. This group is comparable with group I (fig. 3D).

GROUP VII.—Growth response was characterized by continuous axial elongation, the daily increments increasing during the final days of the observed period. There was very good approximation of the original vascular tissues as well as good development of effective new vascular connections similar to those found in group VI (fig. 3A).

Discussion

On the basis of the observed growth responses, the seven groups may be classified into two main categories representing four types. Groups II, IV, and V, which gave a common characteristic in the *ultimate cessation* of the growth rate, belong to types A and B.

Groups I, III, VI, and VII, which have a common characteristic in the *ultimate acceleration* of the growth rate, can be grouped as types C and D. These two ultimate effects can be correlated to some extent with the degree of approximation of the original bundles of the stock and scion, but are more directly correlated with the extent to which new vascular connections between stock and scion have been established by the differentiation of new vascular tissues.

Initial growth was limited in cases where the original bundles were not opposite (type B; group II), and fair to abundant where there was better approximation of these elements (type A; groups IV and V). In both types the ultimate slowing down or cessation of the growth rate can be correlated with failure to develop effective vascular continuity between stock and scion.

In types C and D a similar correlation exists. Initial growth was limited when the original bundles were poorly approximated (type D; groups I and VI), and was rapid when the apposition was good (type C; groups III and VII). In types C and D there was an ultimate increase in the growth rate, and in each there was good development of effective new vascular tissue. The degree of acceleration of the growth rate appeared to be in direct proportion to the amount of effective vascular tissue differentiated.

Another detail was observed, especially in types A and C. The original xylem elements were strongly infiltrated with a heavily staining material, more particularly near the cut surface (fig. 3B).

In attempting to correlate these data in a hypothesis, it becomes evident that it does not support the original contention of WENT (4) that there is "the necessity of cellular continuity between stock and graft to make the latter grow in length." In types A and C there is considerable growth the first three days after grafting. During this period no vascular continuity has been established, and in type A none ever develops. In exceptional cases growth may take place without vascular continuity, therefore, but in such instances there must be good approximation of xylem and phloem of stock to xylem and phloem of scion. Approximation of parenchymatous cells other than those of xylem and phloem alone is insufficient for rapid initial growth. This leads to the conclusion that the growth factors coming from the stock are moving through the vascular

bundles only. If it is assumed that the growth factors are transported, probably through the phloem, with a mass flow under pressure, as MÜNCH (2) supposes, then the observed facts are in harmony.

In exceptional cases there is such good approximation between phloem of stock and that of scion that the contents of the phloem of the stock may pass directly into that of the scion so that the latter can grow. Of course the greater part of the growth substances are lost by leakage along the cut surface. This is suggested in the first place by the reduced growth rate of the scion, which is at most one-third the original rate, and in the second place by the accumulation in the xylem of heavily staining extraneous material which must have been forced into the vessels. Whenever a complete new connection between bundles of stock and scion is established through the differentiation of one or more vascular strands across the cut surface, the flow of materials can follow this path without loss of pressure and growth at the original rate is resumed. A number of details, such as the apparent stopping of the flow a few days after grafting (in types A and C), could be explained with simple further assumptions, but they can better be postponed until more evidence has been collected.

One more fact should be pointed out. The conclusion that the grafts were more likely to take if they started to grow slowly (4) was borne out by this experiment. Of the grafts which grew rapidly the first days after grafting, only 10 per cent ultimately were successful. But of the slow growing grafts, 40 per cent succeeded.

The validity of some of the conclusions based on the foregoing anatomical evidence has been further tested in two additional experiments in which the grafts were made in such a way that better or poorer approximation of the bundles in stock and scion might be expected. The following types of grafts were made, all stems being cut through the third internode (fig. 4).

GROUP I.—Stems were cut and replaced in their original positions with as good approximation of the vascular bundles as possible. In one group (IA) the two ends were pressed together as firmly as the tissues would permit, and in another group (IB) the contact was so slight that the tissues just touched.

GROUP II.—Stems were cut obliquely and replaced on each other after a 90 per cent rotation of the scion. In this case the original bundles did not have very close approximation.

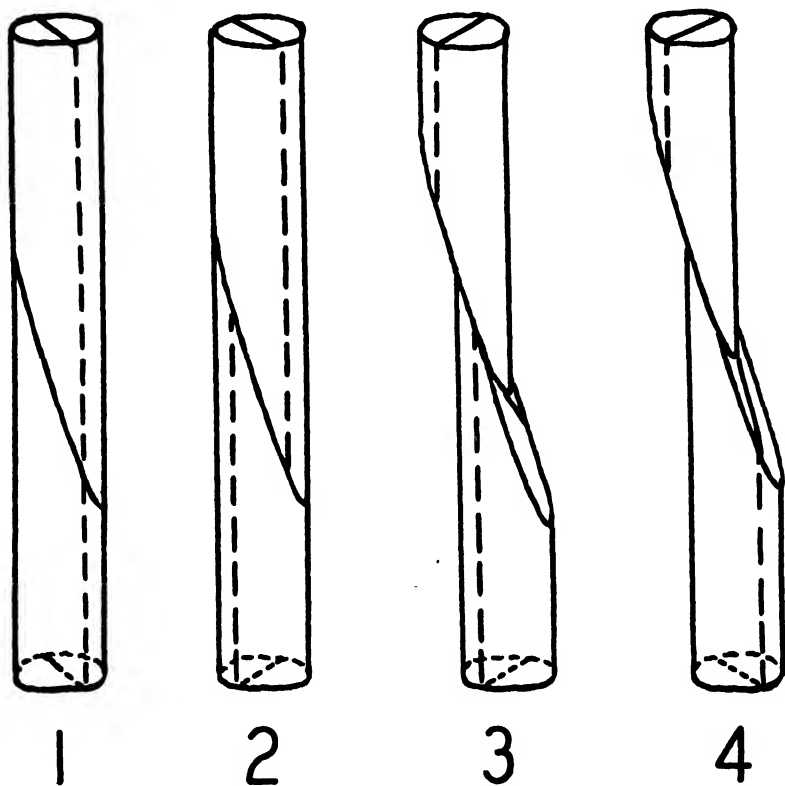


FIG. 4.—Diagrammatic representation of four main groups of grafts in experiments of table 2. Graft 1, stock and scion joined as cut; graft 2, after scion was rotated 90° ; graft 3, after scion was slid halfway across cut surface of stock; graft 4, after additional rotation of scion. Dash line represents plane of the two cortical fiber bundles.

GROUP III.—Stems were cut as in group I, but the cut surfaces of the stock and scion were placed in such a position that there was no good approximation of the vascular bundles.

GROUP IV.—Stems were cut as in group III, but the scion was rotated 90 degrees on its axis and the cut surfaces were placed so that there was no approximation between the original vascular bundles of the stock and scion. Figure 4 diagrams the various types of grafts made. Table 2 gives the results of these experiments.

Growth of group IB was about the same as of IA, except that the maximal growth rate was reached somewhat later than in IA. In both experiments growth of group II was re-established rapidly. Group III was more or less intermediate. Group IV was slowest in starting to grow, having an extra lag of about two days as compared with groups I and II.

Despite these differences, ten days after grafting the same percentage of successful grafts finally took place in all groups and the

TABLE 2

GROUP	PERIOD AFTER GRAFTING (DAYS)												MAXI- MUM GROWTH RATE	MAXI- MUM GROWTH AFTER DAYS	GRAFTS TAKING (%)	ULTI- MATE LENGTH	
	0-2	2-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13						
EXPERIMENT NO. 1																	
Ia	0 7	0 6	0 7	1 7	6 7	15 0	16 2	18 2	19 6	21 0	20 5	39 0	7 5	67	455		
Ib	0 6	0 5	1 2	2 4	4 0	7 0	11 0	15 5	17 2	17 0	17 0	37 5	9 5	65	396		
II	0 8	0 6	1 6	3 4	10 1	15 6	17 6	19 3	23 4	24 1	25 1	38 4	7 5	80	436		
III	0 8	0 6	0 3	2 1	6 4	9 7	11 5	15 5	22 8	19 3	19 0	39 5	8 5	70	440		
IV	0 2	0 5	0 3	1 1	2 9	5 2	8 0	10 1	14 1	16 0	16 6	38 0	10 7	80	417		
EXPERIMENT NO. 2																	
I	1 6	3 0	3 4	13 2	17 4	19 5	13 6	13 0	12 4	11 7	11 0	36 4	7 5	67	362		
II	1 6	2 0	3 6	19 2	21 4	21 5	18 2	13 4	9 0	6 8	4 6	37 8	7 3	75	359		
IV	0 9	1 0	1 0	8 3	10 4	12 2	10 5	10 4	10 5	10 6	34 6	9 2	65	335			

Mean growth rates in mm./day of all grafts of one group (including those which did not resume growth), each group consisting of 20-40 grafts. In the last columns are found the mean maximal growth rate of successful grafts, number of days after grafting this rate is reached, percentage of successful grafts, and mean length of successful grafts at end of experiment.

plants had the same maximal growth rate. This means that ultimately the graft union is equally well established in all four groups.

Only when the approximation of vascular bundles is slight, either as a result of mechanical contact (group IB) or topography (group IV, and to some extent group III), is there a somewhat greater lag between grafting and reaching the maximal growth rate.

Another interesting conclusion, corroborating one drawn from the anatomical investigation, can be reached. Initial growth for the first two day period is least in group IV; is greater but approximately the same in all other groups. This indicates that only with sufficient initial approximation of vascular elements of stock and scion can growth factors move into the scion, and that these move even before new vascular connections are established from the bundles of stock

to those of the scion. Therefore the movement of growth factors of the caline type seems to take place under pressure.

In an earlier paper (4) the conclusion was drawn that the overgrown cut surface, or the graft union, is no impediment to the translocation of food and growth factor, since the growth rate of the grafted shoots was approximately the same as for similar nongrafted shoots. A new experiment confirms this conclusion. A number of Alaska tops were grafted on Alaska stocks. One-half of the tops were grafted directly; that is, with only one graft union. In the other graft a piece of stem was interposed so that there were two graft unions. If the graft union was the factor limiting the growth of the shoot once the graft had taken, there would be a great difference between the two groups, the one with the two graft unions being much inferior to the single grafted peas. However, if the growth of the tops were determined by the base independent of the presence of the graft, both groups might be expected to exhibit equal growth. The results were as follows:

One graft union: Ultimately ten out of twenty grafts were successful and grew at a maximal rate of 26.5 mm. per day, this rate being reached eleven days after grafting.

Two graft unions: Ultimately fourteen out of twenty grafts were successful, and twelve days after grafting a maximal growth rate of 27.4 mm. per day was reached.

In the same experiments, some grafts of Daisy and Alaska tops on Daisy and Alaska bases were made. As in the earlier experiments (4) it was found that the growth rate of Daisy was exactly the same (9 mm. per day) whether grafted on Alaska or Daisy. And the growth rate of Alaska on Daisy was 27 mm. per day. These figures are reported to show that the preceding experiment was a representative one, although the maximal growth rate in all groups was somewhat slower than normal.

The conclusion is clear. Although the maximal growth rate is reached somewhat later in the double grafted stems than in the others, the ultimate growth rates are identical and the percentage of grafts that "take" is at least as good. Some double grafting experiments are now under way to determine what effect an intermediate

stem piece of a different variety may have on the stock-scion relationship (3).

Summary

1. The anatomy and histology of the graft union of etiolated pea stems have been investigated. Five and nine days after grafting, the graft unions were fixed and sectioned. Separation into four types was made between the grafts which grew rapidly or slowly to begin with and those which ultimately did or did not take. The following correlations were found:

2. In the grafts which grew rapidly in the first days, very good approximation between the original vascular bundles of stock and scion was found. When the approximation was poor, the grafts grew slowly to begin with. In the grafts which ultimately took, good development of new vascular connections between stock and scion was found. Such connections were almost or completely lacking in the unsuccessful grafts.

3. To test how far good or poor initial vascular approximation influenced the growth rate of the grafts, some special experiments were carried out which indicated that only the initial growth rate and the period before the scion reaches its maximal growth rate are affected by it. The ultimate maximal growth rate and the percentage of successful grafts were not significantly different in grafts with good or poor approximation. A result corroborating these conclusions was obtained when two graft unions were made instead of one. This also did not alter the maximal growth rate of the scion. Thus in peas the ultimate growth of the scion is not affected by the type of grafting or the graft union.

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COLORIMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF INDOLE(3)ACETIC ACID

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(WITH THREE FIGURES)

Introduction

It has been found that cuttings from many kinds of plants respond, through increased root production, when treated with aqueous solutions of indoleacetic acid that range in concentrations between 0.01 and 0.2 mg. of acid per cubic centimeter (2, 4, 6, 7). To facilitate the study of the absorption of this acid by plants or plant parts from water solution, and to supplement available biological tests (3, 8), it is desirable to have an accurate chemical method with which aqueous solutions of the acid can be analyzed and any amount absorbed be determined by difference.

SALKOWSKI (5) reported a qualitative test in which a nitrite and a mineral acid were used to give a colored solution in the presence of indoleacetic acid (*Skatolecarbonsäure*) occurring among the products of protein putrefaction. He also found that a red color developed when ferric chloride was added to a solution containing this substance in the presence of a mineral acid. Other tests have been proposed but these two have been most commonly used in qualitative determinations of indoleacetic acid. WINKLER and PETERSEN (9) devised a quantitative test for indoleacetic acid, using as reagents copper, glyoxylic acid, and sulphuric acid. This method was also quantitative for the estimation of tryptophane. ZIMMERMAN and HITCHCOCK (10) recently applied WINKLER and PETERSEN's test to extracts from plants that had previously been treated with indolebutyric acid. ALBAUM, KAISER, and NESTLER (11) recently modified the ferric chloride-hydrochloric acid test as a spot test technique and measured quantitatively the penetration of indoleacetic acid into *Nitella* cells.

In the present investigation the reaction of potassium nitrite

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(nitrous acid) and indoleacetic acid was studied in detail with respect to factors that affected the intensity and stability of the color produced. Other colorimetric reactions also studied, but in less detail, included (1) ferric chloride and indoleacetic acid; (2) nitrite and indolebutyric acid; and (3) nitrite and indolepropionic acid. A photometer employing a balanced photoelectric circuit was used. This instrument was equipped with a mercury lamp, the light from which was passed through appropriate filters. All measurements were conducted at a temperature of $24^{\circ}.5-25^{\circ}.5$ C.

Experimental results

NITRITE TEST

In recommended tests for the qualitative determination of indoleacetic acid, concentrated nitric acid is added to a small volume of solution containing indoleacetic acid and potassium nitrite. A red color forms immediately, together with a precipitate which is perceptible in the case of solutions containing more than approximately 0.015 mg. of indoleacetic acid per cubic centimeter of solution. In general the photometric readings of any given solution increased for a period of time to a peak, following the addition of the reagents, and then slowly decreased. The addition of gum arabic as a protective colloid prevented the formation of the precipitate, but did not alter the general behavior of the reaction with respect to color formation. It was thus possible to control the reaction so that clear colored solutions were obtained, and difficulties concerned with the formation of a colloidal precipitate were avoided.

In order to improve the method further, the following factors were investigated individually: (1) amount and kind of acid; (2) amount of nitrite; (3) amount of indoleacetic acid present; and (4) effect of adding the mineral acid at different intervals of time following the addition of the other reagents. The effects of these factors on the intensity and stability of color were measured.

1. ACIDS.—Several mineral acids were used to adjust a known amount of indoleacetic acid solution containing potassium nitrite to a pH of approximately 0.9, and the percentage of light absorbed by different solutions was compared. Seventy-nine per cent absorption was obtained when the acidity of a solution containing 25 cc. of in-

doleacetic acid (0.05 mg. per cubic centimeter) and 0.3 cc. of 0.5 per cent potassium nitrite was adjusted with nitric acid. Under similar conditions, but using sulphuric and hydrochloric acids respectively, the percentage absorption was 78.4 and 80. When solutions were acidified to a pH of 2.5 with either acetic or nitric acid, approxi-

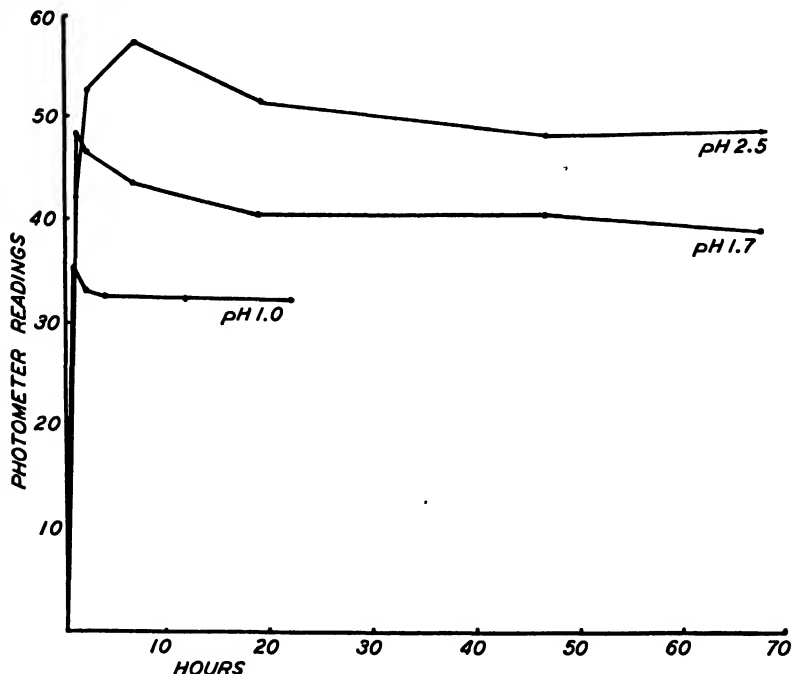


FIG. 1.—Effect of acidity on sensitivity and stability of color formed as result of reaction between indoleacetic acid and nitrite. Time expressed as hours following addition of reagents.

mately equal readings were recorded. Apparently the reaction between indoleacetic acid and nitrite was dependent on the pH of the reaction mixture and independent of the acid anion used, when the acid was added immediately following the addition of the other reagents.

The effect of pH on the time required to obtain equilibrium was studied by adding various amounts of concentrated nitric acid to 50 cc. of a solution containing 0.025 mg. of indoleacetic acid per cubic centimeter, 2 cc. of 0.5 per cent potassium nitrite, and 0.5 cc. of 5 per

cent gum arabic. Figure 1 shows that with increased acidity equilibrium was attained more rapidly than when less acid was used. The percentage absorption of light by solutions at a high acidity, however, was less than that of similar solutions that reacted at a lower acidity. Although increased acidity resulted in lower readings, it offered the advantage that less time was required for the attainment of equilibrium. Within the limits studied, pH had little effect on the stability of the solutions after equilibrium was attained. Maximum readings were obtained when the reaction occurred at a pH of ap-

TABLE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF NITRITE ON SENSITIVITY OF NITRITE TEST FOR INDOLEACETIC ACID. 0.625 MG. INDOLEACETIC ACID USED FOR EACH TEST AND PH OF EACH REACTION ADJUSTED TO 0.98. GUM ARABIC NOT USED

TEST	AMOUNT OF KNO ₂ PRESENT (MG.)	MAXIMUM READ- ING EXPRESSED AS SCALE DIVISIONS	TIME REQUIRED TO REACH MAXIMUM (MIN.)
1.	0 25	87 4	10
2.	0 5	101.0	5
3.	1 0	107.0	4
4.	2 0	99.5	4
5.	3.0	86.0	4
6.	4.0	74.0	3
7.	5.0	61.1	2.5

proximately 2.5, a value well below that of the ionization constant of indoleacetic acid (1).

2. NITRITE.—The effect of nitrite concentration on color intensity was studied by adding various amounts of potassium nitrite to aliquots of solution containing a known amount of indoleacetic acid, and finally adding concentrated nitric acid. Preliminary experiments in which gum arabic was not used showed that the maximum color intensity attained depended upon the amount of nitrite present (table 1). The time required for the attainment of maximum color also depended to some extent upon the amount of nitrite used. Subsequent tests were carried out in which various amounts of 0.5 per cent potassium nitrite were added to 50 cc. aliquots of solution containing 0.5 cc. of 5 per cent gum arabic and 2500 mg. of indoleacetic

acid, and four-tenths of 1 cc. of concentrated nitric acid was finally added. The maximum intensity of color was produced when 0.1 cc. of nitrite was used, but the color was not stable under these conditions (table 2). It was therefore desirable to increase the amount of nitrite to 2 cc. and thus stabilize the system, although a lower intensity of color resulted.

3. INDOLEACETIC ACID.—To determine the relationship between the amount of indoleacetic acid present and the color intensity of the reaction mixture, 2 cc. of 0.5 per cent potassium nitrite, 0.5 cc. of 5

TABLE 2
EFFECT OF DIFFERENT AMOUNTS OF NITRITE ON
SENSITIVITY OF NITRITE TEST FOR INDOLE-
ACETIC ACID. GUM ARABIC USED

0.5% KNO ₂ (cc.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS	
	3 HOURS	20 HOURS
0.05	69 1	58 0
0.1	100 3	90.7
0.5	101 2	88 1
1 0	86 5	82.5
2 0	60.0	61 7

per cent gum arabic, and 0.4 cc. of concentrated nitric acid were added to solutions containing different amounts of indoleacetic acid ranging in concentrations from 0.01 to 0.10 mg. per cubic centimeter. Immediately following addition of the reagents a red color developed in all the solutions. The initial intensity of this color decreased as the reactions came to equilibrium over a period of 2 hours, and in the case of the weaker concentrations, the color changed so that they appeared less red and more yellow, while that of the stronger concentrations remained red. Photometric readings, made with the aid of a filter that transmitted a wave length of approximately 546 mμ, bore a linear relationship to the amount of indoleacetic acid in the various solutions tested when measurements were made after equilibrium had been attained (fig. 2). The solutions were stable and the readings remained constant for 24 hours or longer.

4. TIME INTERVAL BETWEEN ADDITION OF NITRITE AND NITRIC ACID.—Gum arabic and potassium nitrite were added to aliquots of solution containing a known amount of indoleacetic acid. Nitric acid was then added after various intervals of time. A typical red color was developed immediately upon the addition of nitric acid irrespective of the time the indoleacetic acid and nitrite were allowed to react before the nitric acid was added. The color was more intense when the indoleacetic acid and nitrite were allowed to react for a

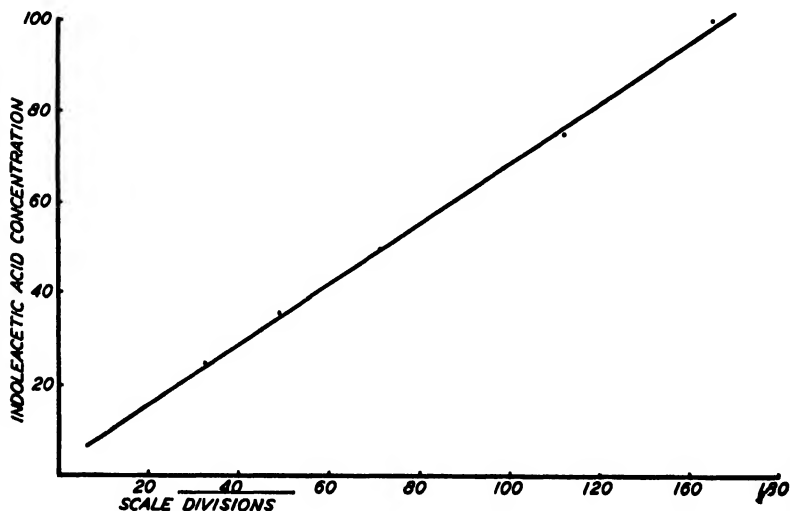


FIG. 2.—Effect of various concentrations of indoleacetic acid upon intensity of color formed as result of reaction of indoleacetic acid, potassium nitrite, and nitric acid. Concentrations of acid expressed as gamma (0.001 mg.) per cubic centimeter in solution tested.

period of time before nitric acid was added, than when it was added immediately (table 3). When indoleacetic acid and nitrite were allowed to react for 30 minutes or more, then added amounts of concentrated acid ranging between 0.1 and 2 cc. had no noticeable effect upon the intensity of color produced. The stability of color, however, was greatest when the acid was added immediately following the addition of nitrite. Stability was considered of greater importance than increased sensitivity and for this reason the acid was added, in subsequent experiments, immediately following the addition of nitrite.

SPECIFICITY OF NITRITE REACTION.— The nitrite reaction was tested for specificity by adding 2 cc. of 0.5 per cent potassium nitrite and 0.4 cc. of concentrated nitric acid to 50 cc. of solutions containing respectively: 0.05 mg. per cubic centimeter of (1) indoleacetic acid, (2) methyl ester of indolebutyric acid, (3) indolepropionic acid, (4) tryptophane, (5) indole, and (6) indolebutyric acid. The test gave a typical cherry red color only in the case of indoleacetic acid and indole, and a yellow color when indolebutyric acid, the methyl

TABLE 3
EFFECT OF TIME INTERVAL BETWEEN ADDITION OF NITRITE AND NITRIC ACID ON INTENSITY OF COLOR PRODUCED. GUM ARABIC AND POTASSIUM NITRITE ADDED TO 50 CC. ALIQUOTS OF SOLUTION CONTAINING KNOWN AMOUNT OF INDOLEACETIC ACID. NITRIC ACID THEN ADDED AT VARIOUS INTERVALS FOLLOWING ADDITION OF THE NITRITE

TIME BETWEEN ADDITION OF KNO_2 AND HNO_3 (MIN.)	23 HOUR READING EX- PRESSED AS SCALE DIVISIONS
0.....	68.1
2 ...	73.5
10 ...	82.0
30 ..	95.1
60.	96.7

ester of indolebutyric, and the indolepropionic acids were used, while tryptophane gave no color.

To determine the applicability of the test to quantitative determinations of indolebutyric and indolepropionic acids, the reagents were added to various concentrations of these acids and the percentage of light absorption measured using a filter that transmitted a wave length of approximately 436 $\text{m}\mu$. Although a detailed study of factors involved in these tests was not made, it is evident that the color intensity varied with respect to the concentrations used (fig. 3).

RECOMMENDED TEST FOR INDOLEACETIC ACID USING NITRITE

The following procedure is recommended for the estimation of indoleacetic acid present in aqueous solution in concentrations ranging from 0.01 to 0.2 mg. per cubic centimeter.

- (1) 50 cc. of indoleacetic acid solution;
- (2) Add 0.5 cc. of 5 per cent gum arabic;
- (3) Add 2.0 cc. of 0.5 per cent potassium nitrite;
- (4) Add 0.4 cc. of concentrated nitric acid immediately following addition of the nitrite.

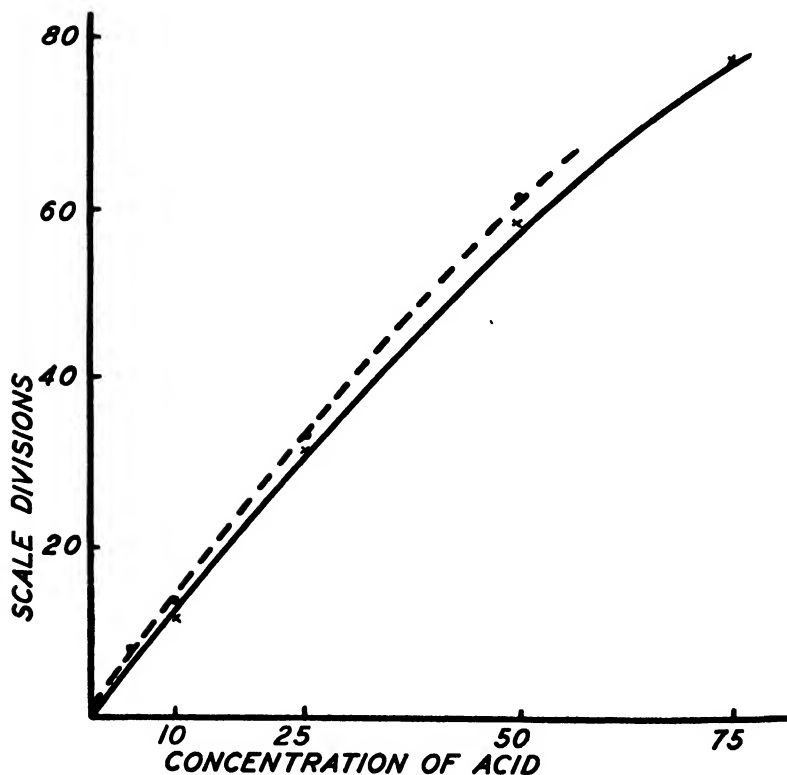


FIG. 3.—Applicability of nitrite test for quantitative determination of indolepropionic and indolebutyric acids in water solutions. Photometric readings of solutions containing indolepropionic (broken curve) and indolebutyric (solid curve) plotted in terms of concentrations of acids expressed as gamma (0.001 mg.) per cubic centimeter of solution.

Shake and allow to stand at room temperature for 2 hours, after which time the color intensity will remain stable for 24 hours or longer.

The percentage of light absorbed by the solution can be measured with the aid of a photometer and compared with that absorbed by a

control solution containing a known amount of acid. With the electrophotometer used, cells 50 mm. in length and having a capacity of 25 cc. have been found convenient for this purpose. An alternative method of estimating color intensity consists of comparing the intensity of color produced through the use of unknown solutions with the intensity produced through the use of known concentrations of acid by means of Nessler tubes. The accuracy of this method has not been investigated.

TABLE 4
ACCURACY OF PROPOSED NITRITE METHOD FOR ESTIMATING
INDOLEACETIC ACID IN WATER SOLUTIONS

DETERMINATIONS	CONCENTRATIONS OF INDOLEACETIC ACID		
	0.01 MG. PER CC.	0.025 MG. PER CC.	0.05 MG. PER CC.
1.....	4 2	21 0	63 7
2.....	4.0	21 5	62 9
3.....	3.4	21.2	63.7
4.....	4.3	21 4	62.9
5.....	4 3	21.4	64 6
6.....	4 4	21.5	64.5
7.....	4.1	21.3	64 0
8.....	4 3	21.3	63 7
9.....	4 4	21 5	64.5
10.....	4 3	21.3	63.7
Average.....	4 17	21 34	63.82
Standard deviation.....	0.0994	0.05213	0.2245
Coefficient of variance.....	2.38	0.24	0.35

The reproducibility of results obtained through the use of the nitrite method proposed was investigated by making replicate determinations on several solutions containing different amounts of indoleacetic acid and estimating the color intensity by means of a photometer. The coefficient of variability was less than 3 per cent for solutions containing as little as 0.01 mg. of acid per cubic centimeter (table 4). The effect of temperature on the results was found to be insignificant over a range from 15° to 30° C. During the use of the nitrite method it was noted that the 0.5 per cent potassium nitrite reagent employed remained stable over a period of 3 weeks, as indicated by frequent titrations.

The particular combination of reagents selected for the proposed method was one of several possible combinations. Other combinations, such as the use of less nitrite and nitric acid, and the addition of nitrite 30 minutes previous to the addition of the mineral acid, or the reduction in volume of all reagents in proportion, might prove advantageous in certain types of investigations when increased sensitivity is desired.

FERRIC CHLORIDE REACTIONS

A red color develops when a mineral acid is added to a solution containing indoleacetic acid in the presence of ferric chloride. A precipitate is not formed and the color varies somewhat with the concentration of mineral acid used. Two methods were found to be usable, one employing hydrochloric acid and iron in proportions similar to those described by ALBAUM, KAISER, and NESTLER (1), and the other, sulphuric acid together with ferric chloride. The former method, which was not studied in detail, was sensitive to concentrations of approximately 0.002 to 0.1 mg. of indoleacetic acid per cubic centimeter (table 5). The latter, which was studied in more detail, was extremely sensitive to small amounts of indoleacetic acid, the minimum total amount detectable being approximately 0.002 mg. A systematic study of the ferric chloride-sulphuric acid method was made concerning the effect of the following factors upon the stability and intensity of color produced: (1) concentration and kind of acid; (2) concentration of iron; (3) concentration of indoleacetic acid.

1. ACID.—A red colored solution was obtained when hydrochloric, phosphoric, nitric, and sulphuric acids were used. The time required for the color to reach a maximum intensity and its subsequent stability varied, depending to some extent upon the acid used. Sulphuric acid was found most suitable as its use resulted in a relatively stable color of high intensity. The ratio between the volume of acid and water present had a marked effect upon the sensitivity of the test, the maximum being attained when the ratio of the volume of acid to the volume of water present was approximately 1:1.5 (table 6).

2. IRON.—One-tenth molecular ferric chloride was used. Volumes of this reagent ranging from 0.01 to 10 cc. were employed to deter-

mine the effect of different amounts upon the sensitivity of the test. The use of relatively small volumes of iron solution decreased the sensitivity. On the other hand, the yellow color of ferric chloride

TABLE 5

EFFECT OF DIFFERENT AMOUNTS OF INDOLEACETIC ACID ON INTENSITY OF COLOR RESULTING FROM ADDITION OF FERRIC CHLORIDE AND HYDROCHLORIC ACID (ALBAUM, KAISER, AND NESTLER). TWENTY-FIVE CC. OF 3 PER CENT FERRIC CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID ADDED TO 25 CC. VOLUMES OF INDOLEACETIC ACID SOLUTIONS. REAGENTS PREVIOUSLY CHILLED TO PREVENT HEATING

INDOLEACETIC ACID PER CC. (MG.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS		
	2.5 HOURS	10 HOURS	24 HOURS
0.100	116.5	114.0	108.5
0.075	109.6	104.5	96.8
0.050	97.2	94.3	82.0
0.025	79.5	75.0	62.0
0.010	56.0	51.7	43.2
0.005	38.0	36.1	30.2
0.002	29.3	26.7	23.8

TABLE 6

SENSITIVITY OF FERRIC CHLORIDE-SULPHURIC ACID TEST AS AFFECTED BY DIFFERENT AMOUNTS OF WATER WITH RESPECT TO VOLUME OF MINERAL ACID PRESENT. MEASUREMENTS MADE 1 HOUR AFTER ADDITION OF ACID

INDOLEACETIC ACID PRESENT (MG.)	VOLUME OF WATER (CC.)	VOLUME OF H ₂ SO ₄ (CC.)	PERCENTAGE LIGHT ABSORPTION
0.1	17.5	10	73.0
0.1	25.5	25	51.7
0.1	45.0	10	8.2

persisted when relatively large amounts of the reagent were used, and in this way interfered with the test by increasing the final color intensity. The volume of reagent required for maximum sensitivity was not sharply critical, however, being approximately 0.5 cc. for the method used.

3. **INDOLEACETIC ACID.**—Solutions containing various amounts of this acid were tested by adding 0.5 cc. of one-tenth molecular ferric chloride and 12 cc. of concentrated sulphuric acid to 18 cc. of solution containing different amounts of indoleacetic acid. Under these conditions photometric readings bear a quantitative relationship to

TABLE 7

EFFECT OF DIFFERENT AMOUNTS OF INDOLEACETIC ACID ON INTENSITY OF COLOR RESULTING FROM ADDITION OF FERRIC CHLORIDE AND SULPHURIC ACID. EIGHTEEN CC. OF SOLUTION CONTAINING DESIRED AMOUNT OF ACID AND 0.5 CC. OF M/10 FERRIC CHLORIDE ADDED TO 12 CC. OF CONCENTRATED SULPHURIC ACID

TOTAL AMOUNT INDOLEACETIC ACID GRAMMA (0.001 MG.)	PHOTOMETRIC READINGS MADE AT DIFFERENT INTERVALS FOLLOWING ADDITION OF MINERAL ACID AND EXPRESSED AS SCALE DIVISIONS		
	1 HOUR	7 HOURS	14 HOURS
100	126 6	68 2	60 7
90	123 4	64 0	54.2
80	115 5	62 5	53 8
70	110.0	56 0	47 8
60	100 0	49 8	42 0
50	92 0	43 8	37 6
40	75 6	37 0	33 4
30	59 0	29 4	25 6
20	40 6	21 2	16 9
15	30.9	16 5	14.9
10	22 0	11.0	9 6
8	17.3	8 0	7.4
6	13 0	5.8	5.5
4	9 0	4 3	3.5
2	4 6	1.8	2.2

the amount of acid present, when total amounts of from 2 to 100 mg. of the acid are used (table 7).

The color resulting from the use of these reagents increased rapidly to a maximum and then decreased slowly in intensity. In the use of the method it is therefore necessary to take into account the time that elapses between the addition of reagents and the estimation of the color intensity. This difficulty can be compensated by including a series of known concentrations which should be treated the same as those containing an unknown amount of indoleacetic acid.

RECOMMENDED TEST USING FERRIC CHLORIDE

The following method is recommended for the estimation of indoleacetic acid when present in total amounts of from 2 to 100 mg.

- (1) Measure out 10 cc. of sulphuric acid (specific gravity 1.84) by means of a graduated cylinder;
- (2) Add 0.5 cc. of M/10 ferric chloride;
- (3) Add 14.5 cc. of distilled water and cool to room temperature;
- (4) Add 5 cc. of aqueous solution of indoleacetic acid.

Shake and cool to room temperature before estimating color intensity by means of a photometer, Nessler tubes, or colorimeter.

Biological application

The methods of estimating indoleacetic acid as presented in this paper were investigated mainly for the purpose of developing a means of determining the amount of indoleacetic acid that cuttings, whole plants, or parts of plants absorb when placed in an aqueous solution of this substance. In preliminary experiments concerning the biological application of these methods, the amount of indoleacetic acid absorbed by geranium cuttings has been measured. Cuttings were made from plants grown under greenhouse conditions by cutting stem segments under water. Their cut ends were immediately submerged to a depth of 2 inches in a solution containing 0.05 mg. of indoleacetic acid per cubic centimeter. Similar cuttings in another group were partially wilted by allowing them to remain out of water for 1 hour before their cut ends were submerged in indoleacetic acid solution. The volume of water and amount of acid absorbed by the cuttings were determined after they had remained in the solution for 3 hours. Turgid cuttings cut under water absorbed more water and indoleacetic acid per unit of time than did those that were cut in air and allowed to become slightly wilted. It is evident that the nitrite test was applicable for measuring the amount of indoleacetic acid absorbed from water solution by the cuttings used.

It should be mentioned that some plants possibly contain substances that, when present in solution, would interfere with the quantitative tests proposed, and this factor should be considered as a possible source of error. The following test was conducted to de-

termine whether substances might diffuse from the cut ends of geranium cuttings or bean cuttings during treatment and thus interfere with subsequent tests made on the solutions to determine the amount of acid absorbed. The cut ends of the cuttings were submerged in 50 cc. of distilled water for a period of 3 hours. A known amount of indoleacetic acid was then added to a volume of the water in which the cuttings had been standing. The resulting solution was finally analyzed by means of the nitrite method described, and the amount of indoleacetic acid present was found to be equal to that previously added. It was evident that the test was unaffected by any substance that diffused from the cut ends of the plants.

Summary

1. Several colorimetric methods were studied to determine their applicability for the quantitative measurement of indoleacetic acid in water solution. These included the reaction between indoleacetic acid and (1) potassium nitrite and nitric acid; (2) ferric chloride and sulphuric acid; and (3) ferric chloride and hydrochloric acid. The nitrite and the ferric chloride-sulphuric acid tests were investigated in detail in order to determine the effect of the different factors involved upon the intensity and stability of the color produced.

2. The nitrite test proved to be applicable for the determination of indoleacetic acid in aqueous solutions having a range of concentrations from 0.01 to 0.15 mg. per cubic centimeter; the ferric chloride-sulphuric acid test was suitable for the estimation of total amounts of from 0.02 to 0.1 mg., under the conditions described. The ferric chloride-hydrochloric acid test was sensitive to essentially the same range of concentrations as that of the nitrite test, but was less suitable as the color produced was not stable.

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RELATION BETWEEN CHROMOSOME NUMBER AND STOMATA IN COFFEA

COARACY M. FRANCO

(WITH TWO FIGURES)

Introduction

RANDOLPH (5) showed the existence of a relationship between the number of chromosomes and the stomata in *Zea mays*, the tetraploid plants having fewer but larger stomata than the diploid ones. SAX and SAX (7), working with *Tradescantia*, also demonstrated the correlation between the chromosome number and the size and number of stomata. It was thought desirable to have similar information concerning the genus *Coffea*. The trees of this genus, part of the collection of the genetics department of this Institute, include a fairly complete polyploid series ($2n = 22, 33, 44, 55, 66$, and 88 chromosomes), and were available for such an investigation.

Technique

METHOD OF COLLECTING.—After trying several methods for counting the stomata, the strip method was selected; that is, the epidermis is stripped off, mounted in a drop of water, and covered with a coverslip. Preliminary observations indicated that there is no appreciable difference in the number of stomata per unit area from different regions in the same leaf; nevertheless, the epidermis was always taken from the median part, midway between the apex and base and the midrib and margin. There is no difference in the number of stomata on the leaves at the tips of branches as compared with the numbers on leaves from the base of the branch, if the leaves observed are mature. The epidermis used, however, was always taken from leaves located at the third or fourth nodes, counting from the ends of the branches. Mature leaves were used in every case.

It was found that leaves taken from the tops of trees have a few more stomata per unit surface than those taken from the bottom; the difference is small but it was thought desirable always to take

the leaves from a point halfway up the tree. After detaching them, the leaves were carried to the laboratory with their petioles in water.

Since there appeared to be a larger variation in the number of stomata between the leaves of any two trees than between the leaves from the same tree, the mean was calculated from thirty leaves, taken if possible from thirty trees of the same variety. When only one tree was available, the thirty leaves were taken from it.

LLOYD'S method (3) was used for measuring the stomata: the epidermis was taken from the leaf and fixed at once in absolute alcohol. Here also the leaves were taken from the greatest possible number of trees and a mean calculated for each variety (or species). The material was collected between 8:30 and 9:30 A.M., when the stomata were open. NUTMAN (4) has shown that on clear days they may close before noon.

The counts were made with a reticulated $7\times$ eye-piece and a $20\times$ objective in a Zeiss microscope. This field area is equal to 0.3217 sq. mm. The epidermis covering the more pronounced ribs of the leaves was avoided because it does not have any stomata.

Measurements were made with a 6a Leitz objective and a $6\times$ micrometric eye-piece. The length of the stoma was measured and its product multiplied by the constant 0.7854 to give the elliptic area of the opening; the results were converted into square microns.

CALCULATIONS.—In order to determine how many counts were needed to give a significant mean, sixty counts were made on *Coffea excelsa* because this particular species seemed to vary considerably with reference to stomatal number. The sixty counts were divided into two columns of thirty each, a mean calculated for each column, and the two means compared by FISHER'S (1) "*t* test." Thirty counts were sufficient to give a significant mean. The coefficient of variability was 17.96. In plants other than *C. excelsa* thirty counts were made and the coefficient of variability calculated; if the latter did not exceed 17.96, the mean was considered to be good.

The *bullata* variety, of the species *C. arabica* (tree no. 28, octoploid), gave a coefficient of variability of 20.90; however, computations similar to those worked out for *C. excelsa* revealed that here the mean based on thirty counts was statistically satisfactory.

All calculations were made for the number of stomata per field of

the microscope and this area converted into stomata per square millimeter. Twenty measurements were found sufficient to give a mean.

Since in some instances more data were needed for some species, and since only measurements made on epidermis collected within the same hour can be compared, it was thought desirable to collect forty leaves and to make measurements from these.

Data

DIPLOIDS

The diploid plants ($2n=22$) showed the greatest number of stomata (haploid plants have not yet been discovered). Several species are known which have $2n$ equal to 22, and among these there is some variation in stomatal number; however, the variation is small compared with the differences found when diploids are compared with polyploids. The variation from species to species among the diploids called for consideration of them in groups. In the following members of the *C. excelsa* group (whose correct nomenclature may be questioned, as the synonymy of *Coffea* badly needs reworking), the species name is followed by the number of stomata per field, then by the number expressing the total of stomata per square millimeter.

C. excelsa group, $2n=22$.—*C. abeokutae* Cramer 81.77, 254.14; *C. dewevrei* De Wild. et Th. Durand 75.23, 233.81; *C. dybowski* Pierre ex De Wild. 80.93, 251.53; and *C. excelsa* A. Cheval. 69.70, 216.63. Total=307.63, or a mean of 76.91 stomata per field. Mean for the number of stomata per square millimeter=239.04.

C. robusta group, $2n=22$.—*C. bukobensis* Zimmermann 107.00, 332.57; *C. quillou* Wester 124.10, 387.70; *C. laurentii* De Wild. 114.50, 355.87; *C. canephora* Pierre ex Froehner 102.33, 318.04; and *C. uganda* Cramer 97.20, 302.10. Mean number of stomata per field=109.03; mean number of stomata per square millimeter=338.86.

C. liberica group, $2n=22$.—Only one tree available at the Institute. Average number of stomata per field=104.87; average number per square millimeter=325.93.

C. congensis group, $2n=22$.—Only one plant available. Number

of stomata per field = 98.00; average number per square millimeter = 304.58.

Kawisari hybrid, $2n = 22$.—Highest number of stomata for *Coffea* occurs here. Average number of stomata per field = 139.17; number per square millimeter = 432.54.

TRIPLOIDS

Only two triploid plants available and these were synthesized by the genetics department of this Institute. Hybrid $45 \times 37-1$ showed 76.90 stomata per field and 239.00 per square millimeter, and hybrid 36×34 1 had 75.90 stomata per field and 235.90 per square millimeter. Average per field = 76.40; average per square millimeter = 237.45.

TETRAPLOIDS

Twenty-four specimens of *C. arabica* gave similar counts. Only one variety of this species, *monosperma*, was found to be exceptional. Its number is not included in the calculation of the mean. The data for these plants are presented in table 1.

PENTAPLOIDS

Only one pentaploid plant was available and it was too small to use.

HEXAPLOIDS

Two varieties of *C. arabica* were available, var. *bullata*, plant no. 133: 33.50, 104.12 and var. *bullata*, plant no. 270 21: 41.13, 127.83. Average stomata per field = 37.31; average number of stomata per square millimeter = 115.96.

OCTOPLOIDS

All are of the *bullata* variety of *C. arabica*. Only one tree showed the expected number of stomata (plant 28-54), and since this number did not agree with those found in the other octoploids it was omitted in calculating the mean. The data for these plants are: plant 28: 43.73, 135.91; plant 28-54: 23.20, 72.10; plant 56 (count made on mutated branches where $2n = 88$): 42.63, 132.49; and plant 210: 41.73, 129.70. The mean for all of these: stomata per square millimeter = 132.71; stomata per field = 42.70.

Table 2, summarizing the data presented so far, shows that an increase in the number of chromosome sets results in a decrease in the number of stomata on the leaves; however, mathematical proof of the correlation cannot be presented on account of the paucity of data.

TABLE 1

VARIETY OF COFFEA ARABICA	NO. STOMATA PER FIELD	NO. STOMATA PER SQ. MM.
typica Cramer (café nacional)	51.43	159.84
typica Cramer, flava form (café amarelo de Botucatú)	50.37	156.55
bourbon	55.27	171.78
bourbon, flava form (bourbon amarelo)	57.30	178.09
maragogipe	49.07	152.51
maragogipe, flava form (maragogipe amarelo)	46.07	148.16
nana*	61.30	190.52
angustifolia	56.03	174.89
bullata (plant 56)†	50.47	156.55
calycanthema*	53.00	164.72
cera*	53.97	167.74
columnaris	49.20	152.91
erecta	52.80	164.10
erecta, maragogipe form	52.43	162.95
goiaba	50.93	158.29
laurina	59.40	184.61
anomala*	47.70	148.25
mokka	62.83	195.27
monosperma	93.90	291.84
murta	57.93	180.05
pendula	53.33	165.75
polysperma	51.30	159.44
purpurascens	55.07	171.16
sempiflorens*	59.60	185.24
Total = 1398 67		
M = 53.79		167.18

* Varieties not yet described.

† Part of this tree mutated to 88 chromosomes. Count recorded here was made from leaves on normal branches (where $2n = 44$).

The diploids have more stomata per surface unit than the triploids, the triploids more than the tetraploids, and so on. The correlation is well marked between the diploid and triploid species examined; however, *C. excelsa* has almost the same number of stomata as the triploid hybrids. This species has, among other characters, coarser leaves than the other diploid species. The average for the triploids was calculated from only two plants. These differed markedly in their appearance, but there is agreement as to stomatal number and the mean is to be taken as significant.

In the tetraploids ($2n=44$) the largest possible numbers were used in determining the mean. With the exception of the var. *monosperma*, all were very uniform with reference to size and number of stomata.

There is some confusion in the octoploids ($2n=88$). Their mean is higher than the one found for the hexaploids, but this difference did not prove to be significant statistically for the calculated $t=1.39$ as against the value given in the table (5.84 for $p=0.01$). Five trees of different genetic constitution were used. Plant 28-54 was the only one that gave the expected results, and as stated previously, this

TABLE 2

COFFEA	NO. OF CHROMOSOMES	MEAN
Robusta group.....	22	338.86
C. congensis.....	22	304.58
C. excelsa group.....	22	239.04
C. liberica.....	22	325.93
Kawisari hybrid.....	22	432.54
C. arabica \times C. canephora.....	33	237.45
C. arabica.....	44	167.18
C. arabica.....	66	115.96
C. arabica.....	88	132.71

was not included in the calculation of the mean. In one tetraploid tree in which there had been a bud mutation, the octoploid leaves gave a different result from the tetraploid ones: for $2n=44$ the count was 156.86, whereas for $2n=88$ it was 132.49. The tetraploids gave the expected result but the octoploid gave a mean higher than was expected. The difference is statistically significant, for calculated $t=4.84$ as against t of the table, 2.57 where $p=0.01$. In spite of this it is clear that the number of stomata decreases as the number of chromosome sets increases.

It is interesting that the size of the leaves does not affect appreciably the number of stomata per square millimeter. For example, the varieties *nana* and *mokka* have a mean length of leaves of 1.10 and 5.0 cm. respectively, as compared with the varieties *maragophe* and *typica*, with an average length of 14.22 and 12.20 cm. respectively. The width varies correspondingly. Although the

difference in leaf size is striking, the number of stomata per square millimeter is not outside the limits expected for the tetraploids.

The leaves taken from the apex of a tree have a few more stomata per unit surface than those taken from the bottom. This is in agreement with the findings of SALISBURY (6) and others.

Discussion

It was noted previously that the size of the stomata does not vary much between varieties of the same species having the same chromosome numbers; consequently, to ascertain whether or not the difference is a real one, a comparison was made between the varieties having the least and those having the greatest number of stomata. This lack of difference is shown by the fact that the means of given pairs of varieties (or species) are not really different on the basis of the t test. In each of the following three pairs the numbers, in the order given, represent the number of stomata per square millimeter; the area in microns; t calculated; and finally, t of the table, $p=0.01$.

First pair, C. arabica, $2n=44$.—Var. *mokka* 195.27, 22.84, 0.53, and 2.57; var. *anomala* 148.25, 25.16, 0.53, and 2.57.

Second pair, C. excelsa group, $2n=22$.—*C. abeokutae* 254.14, 8.96, 1.62, and 2.57; *C. excelsa* 251.63, 11.46, 1.62, and 2.57.

Third pair, robusta group, $2n=22$.—*C. quillou* 385.70, 12.04, 1.80, and 2.57; *C. uganda* 302.10, 9.47, 1.80, and 2.57.

The stomata of one tetraploid, one hexaploid, and one octoploid were measured, all three varieties belonging to the species *arabica*. Again the numbers given are for stomata per square millimeter; area in square microns; t calculated; and t of the table, $p=0.01$. The chromosome number is indicated in parentheses after each name. Var. *cêra* (44): 167.74, 15.63, 1.56, and 2.57; var. *bullata*, plant 270-21 (66): 127.83, 19.25, 1.24, and 2.57; var. *bullata*, plant 210 (88): 129.70, 18.70, 1.24, and 2.57. From the value of t it is concluded that the size of the stomata does not vary between varieties of the same species having different chromosome numbers.

In order to determine whether the size of the stomata varies from species to species, one variety of each species with 22 chromosomes was measured. The numbers given after each name indicate the number of stomata per square millimeter and the size of the stomata

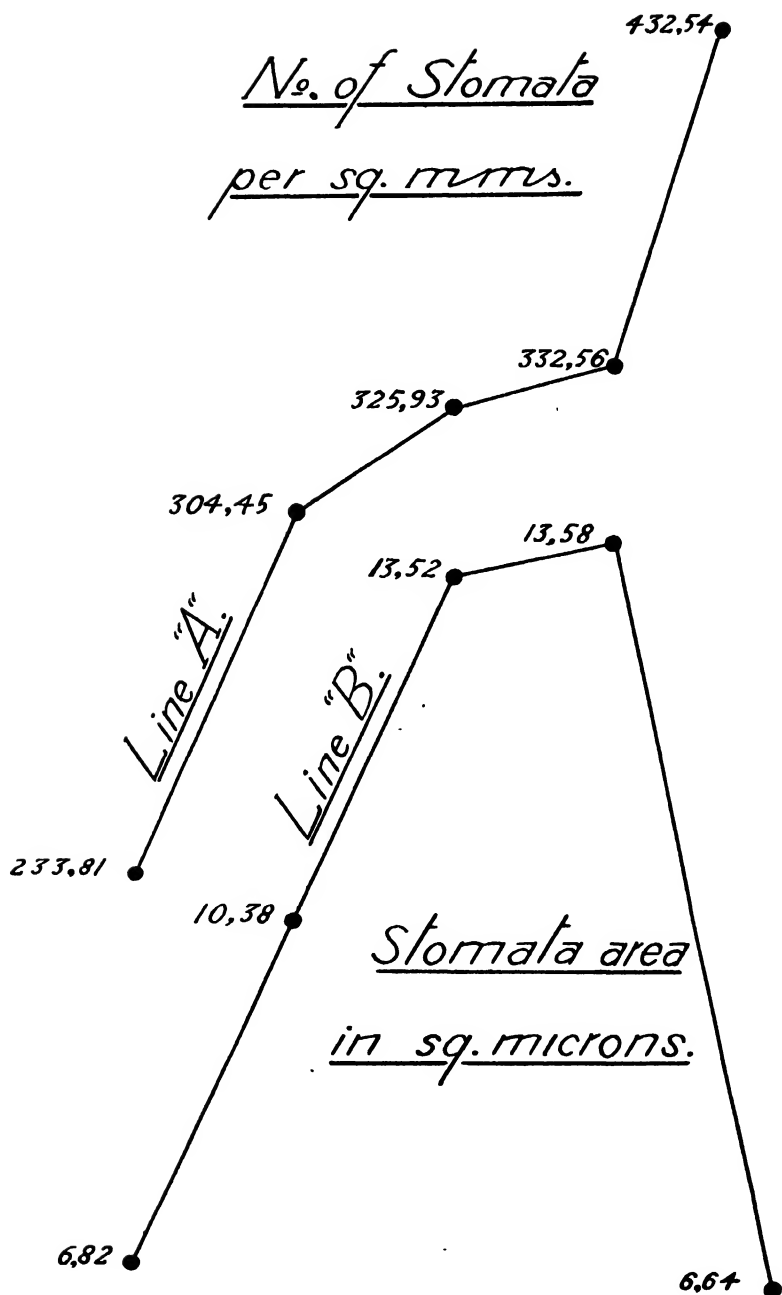
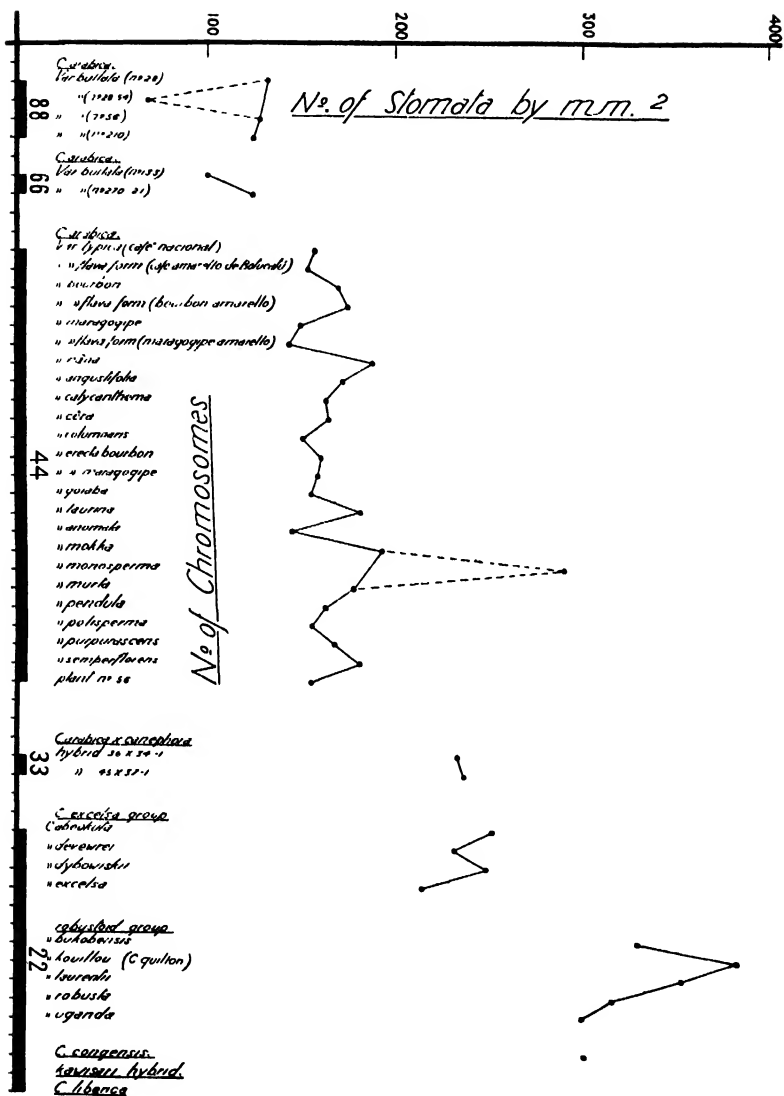


FIG. 1



in square microns. *C. dewevrei*, 233.81, 6.82; *C. bukobensis*, 332.56, 13.58; *C. liberica*, 325.93, 13.52; *C. congensis*, 304.58, 10.38; Kawisari hybrid, 432.54, 6.64.

Figure 1 summarizes the situation. Line *A* represents the data progressively from the smaller to the larger number of stomata in each species. Line *B* is plotted from the values based on the size of the stomata. It should be noted that the difference between the values 13.58 and 13.52 is not statistically significant, hence considering them as the same value the configuration of the curves would not be altered.

Figure 2 indicates that the size of the stomata varies from species to species. The hybrid variety, however, constitutes an exception when it is assumed that the size varies in the same way as the number. The exceptional type can be accounted for by the fact that it is a hybrid. HABERLANDT (2) found many abnormalities when he studied the stomata in forty-five F_1 hybrids. The stomata appear to be restricted to the lower epidermis; none were found in the upper epidermis in any instance.

In addition to the guard cells, the stomatal apparatus has subsidiary cells larger than the former. In general the subsidiary cells contain a large drop of oil which probably has some relation to the stomatal physiology. Green pigmented granules, like chloroplasts, are found in the cells of the lower epidermis in some varieties.

The writer concurs with NUTMAN's statement that the stomata may close before noon on clear days; however, this does not occur if the tree is shaded.

Summary

1. The number of stomata in *Coffea* decreases as the number of chromosomes increases.
2. The size of the leaves has no influence on the number of stomata.
3. The area of the stomatal openings varies with the species and directly with the number of chromosomes.
4. The lower epidermis of several plants seems to have chloroplasts, especially the ones belonging to the group here designated as the *Coffea excelsa* group.
5. In general, the subsidiary cells contain a large drop of oil.

The writer wishes to express his thanks to Messrs. C. A. KRUG, G. P. VIÉGAS, ALCIDES CARVALHO, and A. J. TEIXEIRA MENDES for suggestions made during the course of this investigation, and to Professor W. G. HOUK for help with the English in writing the paper.

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EFFECT OF CALCIUM DEFICIENCY ON RESPIRATION OF ETIOLATED SEEDLINGS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 500

WENDELL R. MULLISON

Introduction

Few references in the literature are concerned with the effect of nutrient deficiency on respiration. SPOEHR and MCGEE (10) worked on the effect of amino acids supplied in nutrient solution to excised *Helianthus* leaves. HAMNER (2) studied the effect of nitrogen supply on the respiration rate of tomato and wheat. GREGORY and SEN (1) reported the effect of nitrogen and potassium on the respiration of excised barley leaves. JONES (3) worked on the respiration of etiolated wheat seedlings as affected by phosphorus. The present work deals with the respiration rate of tops and roots of etiolated plants in relation to calcium supply. Carbohydrate, protein, and fatty seeds were employed in this study.

Material and methods

Pea seeds of the Prince Edward variety, single cross hybrid selected corn obtained from Funk Brothers Seed Company, Bloomington, Illinois, and hubbard squash seeds were used. The weight of each seed varied at most not more than 15 mg. from that of the other seeds employed of the same kind. All seeds were sterilized in sodium hypochlorite, then soaked in distilled water for several hours until definite signs of swelling appeared. At that time they were placed in groups of ten in special beakers filled with sterile quartz sand previously thoroughly flushed with distilled water. Eight beakers were used for each determination. They were placed for germination in the darkroom in which the runs were made. Each day the plants of four beakers were supplied with a complete nutrient solution (7) and the other four with a minus calcium nutrient solution. This latter was the same as the complete nutrient solution except that calcium nitrate was replaced by potassium nitrate. Determinations of the respiratory rate were made when the seedlings were about a week old.

The temperature was kept as close as possible to 29°C ., with a variation of only 1° . The darkroom was lighted by means of a 7 watt red bulb for only the brief time necessary each day to water the plants and to place them in readiness for the respiration determinations.

The apparatus which was built for this work was a modification of the one described in detail by MITCHELL (5), and was based upon the same principles. The amount of CO_2 given off by the plants was determined by the conductivity method. A CO_2 -free stream of air was passed through a closed chamber in which the plants were growing. From here it was bubbled through humidifiers and then drawn through absorption towers. These towers contained 50 cc. of $\frac{1}{10}$ normal lithium hydroxide solution to which had been added 1 per cent butylic alcohol to lower the surface tension. The air flow was regulated to move at the rate of 20 liters per hour through the absorbent, the gases being dispersed as fine bubbles not more than 2 mm. in diameter. The towers were immersed in a water bath kept at a constant temperature that varied not more than 0.01°C . After passage through the absorption towers, the remaining gases flowed through copper tubing into a manifold where the negative pressure was controlled by a valve and indicated by a manometer. From the manifold, a tube led directly to an air pump which created the slight negative pressure required to draw air through the entire system. Sixteen sets of this apparatus were assembled as one unit and operated by a single air pump, accommodating the tops and roots of the eight sets of plants.

The towers were standardized separately by titration with $\frac{1}{10}$ N HCl, using phenolphthalein as the indicator. Thereafter the amount of CO_2 absorbed in the lithium hydroxide solution was determined by using a wheatstone bridge to measure the increased resistance of the solution.

Each chamber in which the determinations were made consisted of two parts, the lower part being the 1.5 liter beaker in which the plants were grown. It was provided with two outlets, one at the center of the bottom and the other one-fourth of the distance from the top. The upper part of the chamber was placed over the tops of the plants at the time of each run. It consisted of 4 inch pyrex tubing sealed at one end and with two side tubes for the incoming and

outgoing streams of air. In order to determine the respiratory rate of the roots as contrasted with that of the tops, they were separated from each other by a seal made of a mixture of paraffin, vaseline, and mineral oil that had a melting point of 40° C. The top part of the chamber was sealed to this paraffin mixture by a paste of clay and castor oil. The two compartments of the chamber were tested by means of a manometer to insure that they were air tight. Before the run the chambers were flushed with CO₂-free air for an hour. Each run involved approximately eighty plants and was of four hours' duration.

Investigation

EXPERIMENTS WITH PEAS

Calcium deficiency became apparent immediately upon germination. The hypocotyls of the minus calcium seeds grew more slowly than those given the plus calcium solution, so that by the end of the

TABLE 1
FOUR-HOUR DETERMINATION ON 7-DAY-OLD PEAS

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	60	3.5
	Roots.....	216	
Minus	Tops.....	32	2.4
	Roots.....	196	

fourth day the radicles of the latter were twice the length of the former. In the minus calcium plants at this time a cessation of growth of the primary root occurred, after which a few laterals grew feebly for a short time. Some of the minus calcium seeds, although producing roots, failed to push their epicotyls above the sand level. The calcium deficiency soon became so severe that necrosis appeared at the tips of the plants. Table 1 gives the data of a representative run.

EXPERIMENTS WITH CORN

Corn was also markedly sensitive to calcium deficiency. Here again the rate of growth of the minus calcium plants was slower than

that of the plus calcium. For the most part in the calcium deficient plants the leaves did not break through the coleoptile, while the plus calcium of the same age had two leaves unfurled. The gross appearance of the corn roots was not noticeably affected by the lack of calcium. Data are shown in table 2.

TABLE 2
FOUR-HOUR DETERMINATION ON 6-DAY-OLD CORN

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	148.8	6.96
	Roots.....	328.0	
Minus	Tops.....	79.2	5.27
	Roots.....	342.4	

EXPERIMENTS WITH SQUASH

Of the three plants used, squash was the slowest to show the effect of calcium deficiency. The rate of growth was retarded by lack of calcium, but necrosis did not set in at once. The length of the hypocotyl was affected while the respiratory rate was but little changed. Table 3 gives the results obtained.

TABLE 3
FOUR-HOUR DETERMINATION ON 6-DAY-OLD SQUASH

CALCIUM	PART OF PLANT	CO ₂ RESPIRED PER 80 PLANTS (MG.)	CO ₂ RESPIRED PER SINGLE PLANT (MG.)
Plus	Tops.....	200	4.4
	Roots.....	152	
Minus	Tops.....	200	4.3
	Roots.....	146	

Discussion

The investigations which have been recorded regarding the function of calcium in plants indicate that it is concerned with permeability, toxic relationships, the actual formation of new cells, and the synthesis of protoplasm. TRUE (11) reported that calcium made

other nutritive elements physiologically available, this effect on permeability apparently being governed by calcium soaps and proteinates. Not only does calcium make other ions available, but in its complete absence plants undergo a leaching of material from their roots. Calcium also aids in preventing toxicity of other elements which in its absence prove harmful (8). NIGHTINGALE has described calcium deficiency symptoms in detail, noting that it first appears in meristematic regions.

With this understanding of the necessity of calcium to the plant, it follows that respiration in its absence would be modified. These results correspond with those obtained by other workers on the effects of nutrient deficiencies upon respiration. HAMNER (2) found that the respiration of tomato and wheat plants decreased when the nitrogen supply was limited. GREGORY and SEN (1) found that the effect of nitrogen and potassium deficiencies was to retard respiration. The latter work was done on excised barley leaves, and not on the entire plant; however, great care was taken to select only leaves of comparable maturity. They emphasize the fact that as individual leaves mature, their respiratory rate changes. JONES reported that the respiration rate of wheat seedlings increased upon the addition of phosphorus.

HAMNER first reported that the respiration rate of the roots and tops of any given plant or group of plants may be very different. Too often in considering respiration that of the tops alone is taken into account, thus neglecting root respiration which in tomato plants supplied with full nutrient solution and wheat seedlings is much more than that of the tops. In the work reported in the present paper it was found that the respiration rate of the roots in etiolated pea and corn seedlings was more than twice that of the tops. This is owing to the fact that there are many actively growing regions in fibrous root systems as contrasted with relatively few meristematic regions in the stem. Where there is less metabolic activity due to the lessened number of actively growing cells, one would expect a slower rate of respiration. In the squash seedlings this great difference in the rate of respiration of the roots as contrasted with that of the tops was not found. The respiration of the roots and tops was about the same, with the tops respiring slightly more. This was probably due to the fact that the cotyledons in

squash come above ground and are the source of food for the seedlings until the leaves are formed. Even so the respiration of the tops was not a great deal more than that of the roots. In corn and pea the cotyledons were not raised above ground, and therefore their respiration was included with that of the roots.

Of the three types of seeds (carbohydrate, protein, and fatty) of the same age, the seedlings which were of the high carbohydrate type respired most, the protein type respired least, and the fatty type was intermediate. Fatty seeds may absorb large quantities of oxygen in the formation of carbohydrates from fats during germination.

Although when roots and tops were separated, the roots of the minus calcium corn plants respired more than those of the plus calcium, the total respiration of the minus calcium plants was always less than that of the plus calcium. At the present time this increased respiration of the minus calcium corn roots presents an interesting unsolved problem. In this connection it may be noted that corn is a member of the grass family, which has been reported to require relatively little calcium (9). GREGORY and SEN reported a slightly increased rate of respiration due to a moderate potassium starvation, and only when the starvation became acute did the rate fall. If the roots do not suffer from calcium deficiency and are normal, however, their respiratory rate should be the same as the controls, whereas actually it exceeded them. In addition it is difficult to explain the severe minus calcium symptoms shown by the tops.

The effect of lack of calcium on squash is the least marked of the three types. Although minus calcium plants were much smaller in size, their respiration was approximately the same as those supplied with complete nutrients. There were few signs of necrosis in the minus calcium plants. Even though the plus calcium ones were larger, their respiration was but slightly more. It may be that the minus calcium squash plants had not yet begun to show a severe calcium deficiency, there having been enough available calcium stored in the seed to serve for immediate needs. This stored supply of available calcium probably varies with the type of seeds used, which may account for the difference in the response of the three kinds used in this work.

These results of calcium deficiency on respiration may be interpreted by considering lack of calcium a limiting factor in anabolic

plant processes; hence with fewer cells and less protoplasm, a lowered respiration rate must occur.¹

A fact worthy of note was the effect on growth of the seedlings immediately after germination when supplied with the minus calcium nutrient solution. This caused a decided decrease in rate of development that was most pronounced in the case of pea. Pea seeds grew and formed radicles approximately 2 inches long, and small epicotyls, after which growth stopped and was not appreciably resumed. Whether this is due to the toxic action of other elements in the absence of calcium, which seems most likely, or to the effect on permeability or its lack in anabolism is not definitely known. Pea seeds contain calcium, but perhaps not much is available for germination. MAQUENNE and DEMOUSSY (4) reported finding a stimulating effect of calcium on pea seed germination. NIGHTINGALE (6) pointed out the extreme immobility of bound calcium in tomato plants. He also stressed the fact that when new cells are formed, calcium must be present, not only for the development of the middle lamella, but also for the synthesis of protoplasm. Since in seeds the amount available for use is small, much of the calcium necessary for the developing of new tissue from nearly the beginning must come from outside the seed. This factor, of course, would probably vary greatly with different types of plants.

Summary

1. The effect of calcium deficiency upon the rate of respiration of etiolated seedlings was studied. Seeds used in this study were pea, corn, and squash, examples respectively of the three general classes of seeds: protein, carbohydrate, and fat. The plants were divided into two parts, the tops and the roots, and the respiration of each was studied separately.

2. Total respiration under conditions of plus and minus calcium nutrition was highest in the corn seedlings, least in the pea seedlings, and intermediate in the squash.

3. Total respiration of the minus calcium plants was in every case less than that of the plus calcium plants.

¹ Work on calcium nutrition of the bean plant (GAUCH, H. G., Responses of the bean plant to calcium deficiency. In Press.), done subsequent to this investigation, shows that with plants given a minus calcium nutrient solution, the toxic effects are due to the other ions in the absence of calcium.

4. Lack of calcium was most noticeable in the greatly lowered respiration of the minus calcium tops as compared with that of the plus calcium tops.

5. Calcium deficiency lowered the respiration of the roots of pea and squash, but increased the root respiration of corn.

6. Roots of pea and corn respired more than twice as much as did their tops.

7. Respiration of the roots and tops of squash was about equal, although the tops respired more than the roots.

The writer is indebted to members of the department of botany of the University of Chicago for helpful suggestions made during the course of this study.

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VITAMIN B₁ IN RELATION TO MERISTEMATIC ACTIVITY OF ISOLATED PEA ROOTS

FREDRICK T. ADDICOTT

(WITH ELEVEN FIGURES)

Introduction

During the last five years the growth of excised roots has been studied intensively, particularly with regard to nutritional requirements. It has been shown by BONNER (1), and simultaneously and independently by ROBBINS and BARTLEY (7), that in addition to the nutrients of salts and sugar, vitamin B₁ is essential to the growth of excised roots. The vitamin affects not only the growth in length of the roots but also their general morphology. Roots grown with an excess of vitamin B₁ are so different in external appearance from those whose growth is limited by the available vitamin B₁ as to suggest the possibility of considerable difference in the internal structures as well. The present paper reports some effects of vitamin B₁ on excised pea roots grown in vitro.

Material and methods

The methods used were essentially those described by BONNER and ADDICOTT (3). Sterile technique was used throughout the handling of the cultures. Pea seeds (*Pisum sativum*) of the variety Perfection were sterilized to free them of mold spores and bacteria and were germinated in petri dishes at 25°C. At the end of two days, when the primary roots were about 1 cm. long, tips approximately 5 mm. long were removed by means of a surgical scalpel and placed in petri dishes containing 20 cc. of liquid culture medium. This medium contained mineral nutrients and sucrose, to which was added vitamin B₁ in certain cases (table 1). After the roots had grown in the medium for one week at 25°C., the tips were removed and transferred to fresh medium. These tips were always cut 1 cm. long so that the remaining basal portion of each root could be measured. This process of subculture was repeated weekly.

Added vitamin B₁ has no effect on the growth rate of the pea root

during its first week in culture, and consequently was always omitted from the medium during this period. As BONNER (2) has shown, the freshly excised pea root tip contains sufficient of the vitamin (and presumably of other growth factors) to maintain growth during the first week. However, the vitamin must be added to maintain growth in the later subcultures. Since roots to which the vitamin was not supplied ceased growth completely by the end of the third week, the material used in this investigation was not kept in culture longer than this time. At the end of each week, growth was measured and tips were fixed for cytological study. Roots from cultures which had

TABLE 1

BASIC MEDIUM FOR EXCISED PEA ROOTS

CONSTITUENT	CONCENTRATION (MG./LITER)
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	36
KNO_3	81
KCl	65
KH_2PO_4	12
Fe-tartrate	1 5
Sucrose	4%
Vitamin B ₁ (when indicated)	0.1 mg./liter

been maintained up to three weeks without added vitamin B₁ were compared with roots from cultures which had received additional vitamin B₁ during the second and third weeks.

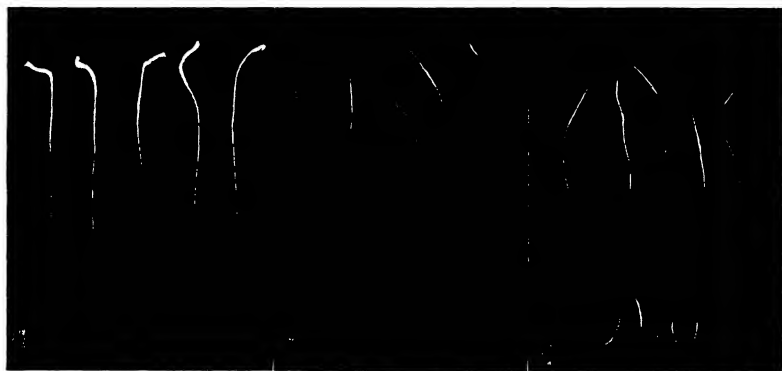
Root tips were fixed in Navashin's solution. In order to eliminate any variation which might be due to diurnal fluctuation of the rate of mitosis, roots were always fixed at noon. Dehydration was accomplished by means of the tertiary butyl alcohol method and material imbedded in a rubber-paraffin mixture. Iron haematoxylin was used as the nuclear stain and fast green as the counter stain.

Investigation

The growth habit of excised roots is indicated by figures 1-3, which are taken from shadow photographs of roots at the end of the first, second, and third transfers. Roots after one week in culture show characteristically a swollen base, but at the end of succeeding transfers the root diameter is more uniform although there is a grad-

ual thickening toward the base. Figures 1-3 show also that the addition of vitamin B₁ to the culture medium results in sustained growth in length of the root. Roots supplied with the vitamin are smooth and white with a slightly yellowish meristematic region when observed at the end of a transfer period. The roots not supplied with vitamin B₁ are short, brownish, and irregularly swollen.

Sections of root tips of seedlings grown for one week in garden soil in the greenhouse were used as a basis for comparison with the sections of excised roots. Roots at the end of the first week in culture



FIGS. 1-3.—Shadow photographs of excised roots, taken at end of each weekly transfer. Fig. 1, end of first week, not supplied vitamin B₁. Fig. 2, end of second week, supplied vitamin B₁, above; not supplied, below. Fig. 3, end of third week, supplied vitamin B₁, above; not supplied, below. $\times \frac{1}{2}$.

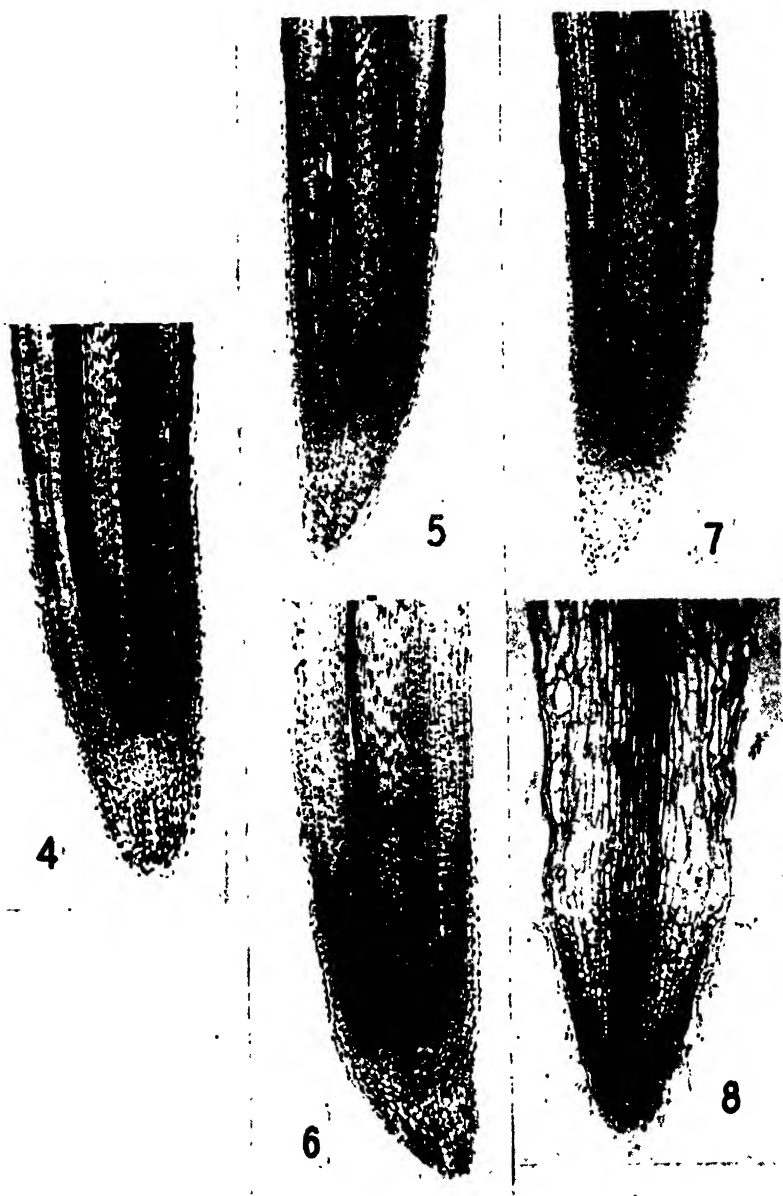
and from later transfers which had received vitamin B₁ appeared in most respects to be closely similar to the seedling roots. They were thinner in diameter, a difference brought about primarily by a decrease in the number of axial rows of cells in the excised roots. Table 2 gives the results of counts of the number of cells along a diameter of a transverse section through the region of elongation. This is equivalent to the number of axial rows of cells which would be found in a median longitudinal section of the root. This table shows that as the roots grow in length during the first three weeks fewer rows of cells arise in the meristem. In cultures to which vitamin B₁ has not been added, and which have correspondingly grown but little in length, this effect is not marked.

The most striking microscopic differences between roots grown with and without the addition of vitamin B₁ are those to be observed in the meristematic region. Roots supplied with vitamin B₁ are essentially normal and possess an extensive and active meristem at the end of the third week. In roots which have not been supplied vitamin B₁ the meristem shows a marked reduction in both extent and activity after three weeks, and a large proportion of the cells which were originally densely filled with cytoplasm now are highly vacuolated. These differences can readily be seen in the photomicrographs of this region which illustrate the effect of vitamin B₁ on the meristem of the roots (figs. 4-10).

TABLE 2
NUMBER OF AXIAL ROWS OF CELLS IN PEA ROOTS GROWN
UNDER VARIOUS CONDITIONS

SOURCE OF ROOT TIP	TIME OF COUNT	NUMBER OF CELLS ON DIAMETER OF TRANSVERSE SECTION OF ROOT	
		NOT SUPPLIED B ₁	SUPPLIED B ₁
Seedlings:			
Primary roots.....	End of first week	48.5 ± 2.8
Secondary roots.....	End of first week	39.8 ± 0.96
Excised roots.....	End of first week	41.1 ± 0.47
Excised roots.....	End of second week	42.0 ± 2.2	39.5 ± 1.0
Excised roots.....	End of third week	39.4 ± 1.4	36.3 ± 0.57

Although vitamin B₁ exerts a marked effect on meristematic activity, roots deficient in the vitamin are nevertheless unaffected in certain other respects. Cell elongation and maturation of the cells arising from the meristem continue to completion. Since very few new cells are formed after the roots become deficient in vitamin B₁, nearly all the cells of these tips are mature. The walls of mature cells are well developed in roots cultured for three weeks without the addition of vitamin B₁. Annular and spiral thickenings of the xylem vessels are laid down, and have frequently been observed within a distance of a few cells from the short meristematic region. The unusual development of the vacuoles should also be mentioned, since this process has attracted much attention; for example, GUILLIERMOND (5). After roots have grown for three weeks without the addition of vita-



FIGS. 4-8.—Longisections of root tips taken at end of each week in culture. Fig. 4, end of first week, not supplied vitamin B₁. Fig. 5, end of second week, supplied vitamin B₁. Fig. 6, end of second week, not supplied vitamin B₁. Fig. 7, end of third week, supplied vitamin B₁. Fig. 8, end of third week, not supplied vitamin B₁. $\times 59$.

min B₁, there are large vacuoles in the cells of the meristem in the region from which the cortical elements are derived. In these same meristems, the cells from which stelar elements develop appear more like those in the seedling roots, although these cells are greatly reduced in numbers. The vacuoles remain small in all the cells of the meristem when vitamin B₁ is added to the culture medium. That the cells of roots deficient in vitamin B₁ are metabolically active is also shown by the fact that they store starch abundantly. The deposition



FIGS. 9, 10.—Root tips at end of third week in culture. Fig. 9, apical portion of meristematic region of root supplied vitamin B₁. Fig. 10, meristematic region not supplied vitamin B₁. $\times 114$.

is greatest in the more proximal regions of the roots, but starch grains, like the vacuoles, are conspicuously present even in cells adjacent to the meristem. These points can be seen to a limited extent in figures 4-10.

In order to obtain a quantitative relation between growth rate and meristematic activity of roots in culture, counts were made of the number of mitotic figures to be found in the meristem of median longitudinal sections of roots subjected to the different treatments. This count is a function of the mitotic frequency. The length of the apical meristem was also measured. These results, together with the growth rates of the corresponding roots, are summarized in ta-

ble 3. They are also presented graphically in figure 11, where for ease of comparison the growth rate, the number of cell divisions, and the length of the meristematic region of the roots at the end of the first transfer are taken as 100 per cent in each case.

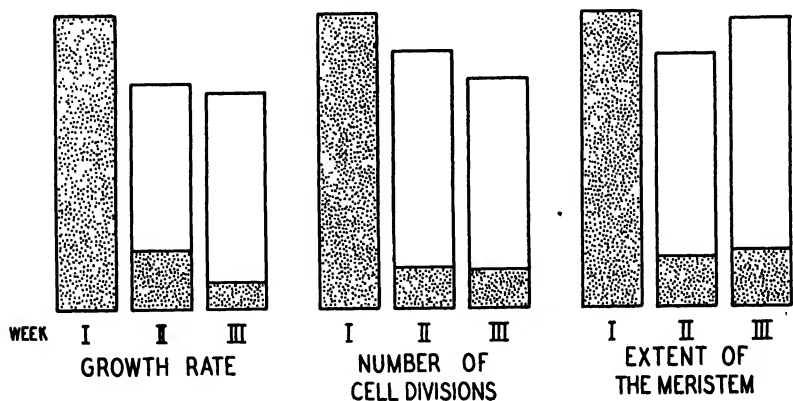


FIG. 11.—Diagram comparing growth rate, number of cell divisions, and extent of meristem of root tips studied, from data of table 3. In each case the value for the first week was taken as 100 per cent. Stippled areas correspond with roots not supplied vitamin B_1 and are superimposed upon clear areas representing roots supplied vitamin B_1 . No roots supplied vitamin B_1 during the first week.

TABLE 3
COMPARISON OF GROWTH RATE, CELL DIVISIONS, AND EXTENT OF
MERISTEMATIC REGION IN EXCISED PEA ROOTS

	FIRST WEEK	SECOND WEEK		THIRD WEEK	
	MINUS VITAMIN B_1	MINUS B_1	PLUS B_1	MINUS B_1	PLUS B_1
Growth rate (mm./week)	59.2	12.0	45.6	5.6	43.5
No. of cell divisions*	18.0	2.5	15.7	2.4	14.0
Extent of meristem (mm.)†	1.55	0.27	1.33	0.30	1.52

* Because of the difficulty of identifying with certainty the early prophase and late telophase stages, only metaphase and anaphase stages were counted. Figures are a measure of the number of cell divisions per median longitudinal section.

† These figures indicate linear extent of apical meristematic activity in root tip. Distance measured was from tip of meristem to last observable mitotic figure which was definitely a part of the apical meristem.

It is now well established that vitamin B_1 may be regarded as a hormone of root growth. It has been shown (4) that vitamin B_1 is produced by leaves in the light and is transported to the roots, where

it affects growth in length. From the preceding observations it is evident that the effect on root growth is in part (and perhaps largely) attributable to an effect on meristematic activity. It will be of interest to determine whether the effect of vitamin B₁ on meristematic activity is in turn attributable to the co-carboxylase function of the vitamin (6).

The effects of vitamin B₁ are thus quite different from those of the auxins which primarily affect cell elongation. With isolated roots there is a close positive correlation between the supply of vitamin B₁ and growth rate, number of cell divisions, and extent of the apical meristem. The effect of vitamin B₁ as a hormone of root growth is thus correlated with the maintenance of normal meristematic activity.

Summary

1. The action of vitamin B₁ as a growth hormone of roots is through an effect on meristematic activity rather than on cell elongation, which is the primary effect of the auxins.

2. Cell elongation, differentiation, and maturation proceeded normally in the roots to which vitamin B₁ was not supplied, as far as could be observed, even though meristematic activity was greatly reduced.

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VITAMINS AND THE GERMINATION OF POLLEN GRAINS AND FUNGUS SPORES

WILLIAM C. COOPER

(WITH TWO FIGURES)

Introduction

Various workers have shown that the addition of extracts of fresh plant tissues of various sorts induces the germination of pollen grains and fungus spores. KNOWLTON (4) observed that the percentage germination of *Antirrhinum* pollen was greatly increased by the addition of minute amounts of crushed stigma. WILCOXON and MCCALLAN (10) found that the addition of small quantities of tomato, orange, apple, or pear juice and aqueous extracts of potato tubers resulted in nearly 100 per cent germination of spores of *Penicillium*, *Glomerella*, *Botrytis*, and *Sclerotinia*. A water extract of sterilized yeast was likewise found to induce germination of pollen (2) and fungus spores (10).

It is now generally known that vitamin B₁, vitamin B₂, vitamin B₆, nicotinic acid, and a complement of amino acids are among the constituents of yeast extract. And of greater interest are the recent findings of BONNER (1) and ROBBINS and BARTLEY (5) that the beneficial effect of yeast extract on growth of excised roots in vitro is because of its vitamin B₁ and amino acid content. It would therefore seem logical to expect that the stimulating effect of crude extracts on pollen and fungus spore germination might be attributed at least in part to their vitamin content. It was with the hope of identifying known chemical substances, such as the vitamins, concerned with pollen and fungus spore germination that the present work was undertaken.

The pollen used in this investigation was obtained from four horticultural varieties of *Carica papaya* which were inbred by sib-pollination for a number of years by TRAUB and ROBINSON (9). The spores used consisted of *Colletotrichum gloeosporioides* from the avocado and papaya, and *Penicillium italicum* from the orange.

Water solutions of several of the vitamins and a number of other

substances were tested individually for effect on germination by the van Tieghem hanging drop technique. All substances tested, except lactoflavin (vitamin B₂ or G), were synthetic crystalline preparations. The lactoflavin preparation was obtained from natural sources of lactoflavin and probably contained appreciable quantities of impurities carried over from the original material. A synthetic crystalline preparation of lactoflavin was obtained after the completion of most of the experiments reported herein, and was tested for its effect on germination.

Each experiment contained a distilled water control which invariably showed less than 5 per cent germination. TRAUB and O'RORK (8) have shown that papaya pollen germinates satisfactorily in a 0.75 per cent agar and 4 per cent sucrose medium. Accordingly this medium was carried along as a check in some of the experiments, in order to compare the present results with those of the past. Agar was not mixed with the substances being tested because it is a natural product consisting of a mixture of substances.

In the inoculation of the hanging drop in the spore experiments, a small number of spores were carried over from sterile cultures on the point of a needle. Distilled water controls did not germinate if care was exercised to avoid carrying over any of the substratum during the inoculation process.

A preliminary survey of the relation of time to percentage germination in the test solution showed that maximum germination of pollen was nearly always obtained after 4 hours and maximum germination of spores after 20 hours. Accordingly these time intervals were used uniformly throughout the investigation.

Investigation

POLLEN OF CARICA PAPAYA

It has recently been reported by DANDLIKER, COOPER, and TRAUB (3) that the addition of thiamin (vitamin B₁) to the Traub and O'Rork sugar-agar media causes a slight increase in the percentage germination of pollen of the Florida and Fairchild varieties of *Carica papaya*, as compared with that obtained on sugar agar without the addition of B₁. No increase, however, was obtained for the Orlando variety. Vitamin B₁ has now been tested without the sugar-agar

supplement and has been found to have some activity (tables 1 and 2). Just as with the earlier experiments, the maximum response was

TABLE 1
SUBSTANCES FOUND ACTIVE IN INDUCING GERMINATION
OF ORLANDO PAPAYA POLLEN

SUBSTANCE	EFFECTIVE CON- CENTRATION (GAMMA PER CC.)		MAXIMUM PERCENTAGE GERMINATION*			
	MIN.	MAX.	OCT. 11	OCT. 13	OCT. 15	OCT. 24
Distilled water	0	2	0	0
Lactoflavin† (vitamin B ₂ or G)	10	50	82	78	74	67
Ascorbic acid (vitamin C) .	50	100	96	80	55	56
Thiamin (vitamin B ₁)	25	100	11	15	10
Nicotinic acid	75	100	4	12	11
Indole(3)acetic acid	25	100	37	8	33	7
Histidine-hydrochloride . . .	50	200	23	41	40
Cysteine-hydrochloride . . .	50	100	12	49	45
Glutamic acid hydrochlo- ride	50	100	28	30
Lysine-dihydrochloride . . .	50	200	45	..	35

* Averages of four determinations.

† Natural preparation.

TABLE 2
EFFECT OF DIFFERENT MEDIA ON GERMINATION OF POLLEN
OF FOUR VARIETIES OF PAPAYA

VARIETY	PERCENTAGE GERMINATION IN MEDIA* (AVERAGE OF EIGHT DETERMINATIONS)					
	WATER	THIAMIN	LACTO- FLAVIN†	ASCORBIC ACID	INDOLE(3)- ACETIC ACID	0.75% AGAR PLUS 4% SUCROSE
Orlando	3	10	78	76	17	49
Florida	0	52	78	62	39	61
Fairchild	0	12	75	72	12	71
Betty	0	35	64	56	7

* Concentration 100 gamma per cc. in all cases except lactoflavin which was 50 gamma per cc.

† Natural preparation.

obtained with the Florida variety and the minimum with the Orlando variety.

In addition to thiamin, a number of other substances were found to be active in inducing germination. Table 1 shows that lactoflavin (natural product) and ascorbic acid were highly effective. Both showed considerably higher percentage germination than thiamin or any of the other substances tested. Also the percentages obtained were higher than those for the sugar-agar medium (table 2). The maximum and minimum effective concentrations of lactoflavin

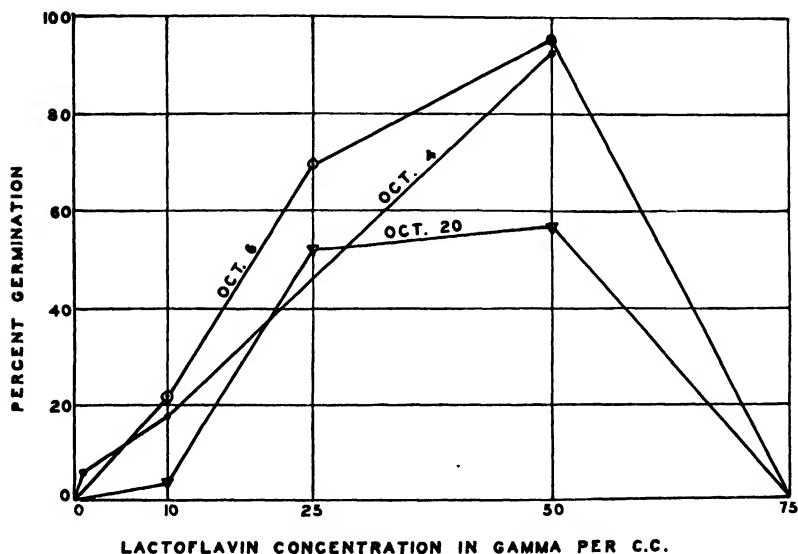


FIG. 1. Effect of lactoflavin (natural product) on germination of pollen of *Carica papaya* var. Orlando (Hort.).

were 10 and 50 gamma per cc. and those of ascorbic acid were 50 and 100 gamma per cc. (figs. 1, 2).

It is noted from table 2 that lactoflavin and ascorbic acid caused high germination percentages for all varieties tested, that of the Orlando variety being as high as or higher than that for the Florida variety. Thus the lactoflavin and ascorbic acid reactions differ from the thiamin reaction, not only in giving greater germination but also in giving nearly equal germination for the various varieties.

The other compounds listed as being effective in inducing germination include nicotinic acid, indole(3)acetic acid, histidine hydrochloride, cysteine hydrochloride, glutamic acid hydrochloride, and

lysine dihydrochloride. It is seen that the amino acids in this group are all hydrochlorides. Other amino acids such as leucine, arginine, valine, asparagine, tryptophane, glycine, alanine, and tyrosine which contain a free NH_2 group were found to be practically inactive under the conditions of these experiments.

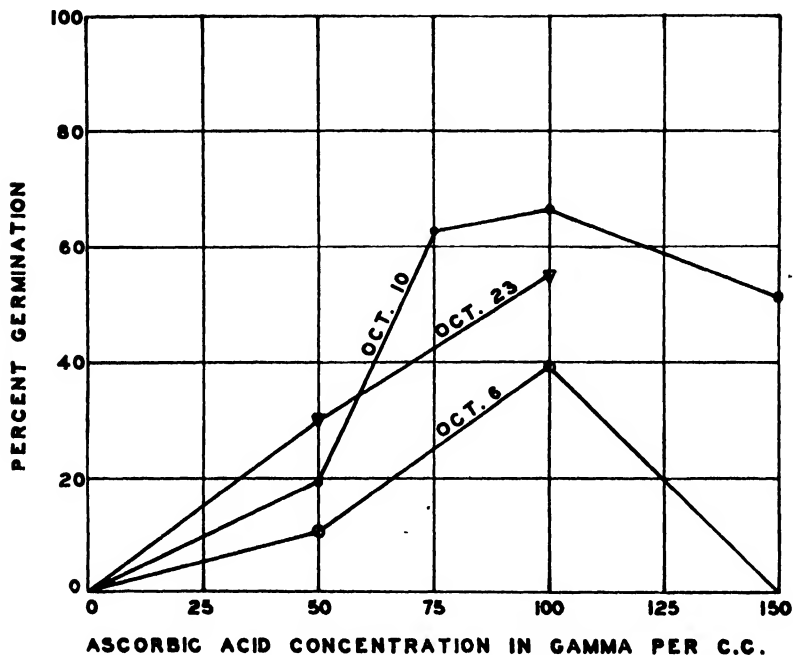


FIG. 2.—Effect of ascorbic acid on germination of pollen of *Carica papaya* var. Orlando (Hort.).

All these observations suggested that the activity of this group of compounds was caused by a pH effect and that any acid might produce the same effect. This was found to be the case when germination percentages comparable with those obtained by indole(3)acetic acid and the amino acid hydrochlorides were obtained with hydrochloric acid. Acetic, benzoic, and malic acids were also found to be slightly active.

The fact that substances in the first group (lactoflavin and ascorbic acid) gave considerably higher and somewhat more regular germination percentages than the substances of the second group would suggest that these two substances may possibly exert a greater

specific effect on germination than that of influencing the pH. It is possible that the lactoflavin preparation, which was a natural product, contained some impurity that was active in inducing germination. A 500 gamma per cc. solution of the natural product was analyzed spectroscopically² and found to contain 1.7 gamma per cc. of boron and traces of aluminum, iron, manganese, and magnesium. It has been reported in recent years that boron is essential to the germination of many kinds of pollen (6). Boron in the form of sodium borate has been tested for its effect on papaya pollen and found to induce germination at concentrations ranging from 0.001 to 1 gamma per cc. It would appear that the response obtained from the natural lactoflavin preparation might be due in part to the presence of boron as an impurity.

Sometime after the completion of these experiments, a synthetic preparation of lactoflavin was tested for its effect on pollen germination and was found to be active. It was, however, only about half as active as the natural product. Apparently lactoflavin does induce pollen germination, but the response obtained from the natural preparation might have been due to a combined effect of lactoflavin and boron.

As a matter of record it is well to mention that carotene (pro-vitamin A), vitamin B₆, and α -tocopherol (vitamin E) were tested for their effect on pollen germination and were found to be without effect. Also sucrose, which is a constituent of most pollen germination media, was found in these experiments to be without activity for 1, 2, 5, 10, and 20 per cent solutions. Sucrose added as a supplement to lactoflavin caused no increase in percentage germination but caused a considerable increase in the length of the pollen tubes.

SPORES OF COLLETOTRICHUM GLOEOSPORIODES

Preliminary experiments on the effect of the various substances on germination of spores of *Colletotrichum gloeosporioides* gave very conflicting results. The first trial showed that thiamin was effective; a second and third trial showed that it had no effect, while lactoflavin (natural product) was quite active in inducing germination. These conflicting results were understood when it was found that the na-

² Spectroscopic analysis was made by Dr. B. C. BRUNSTETTER of the U.S.D.A. Horticultural Research Station, Beltsville, Md.

ture of the growth medium from which the spores were taken influenced materially the response induced by various germinating media. When spores were taken from cornmeal-agar cultures, lactoflavin and a number of other substances, including thiamin, nicotinic acid, ascorbic acid, carotene, α -tocopherol, malic acid, inositol, pimelic acid, tryptophane, leucine, glycine, asparagine, arginine, glutamic acid hydrochloride, and histidine hydrochloride, would induce germination. On the other hand, when spores were taken from oatmeal-agar cultures, only lactoflavin was effective in inducing germination. Thus in the first preliminary experiment thiamin was active in inducing germination because the spores were taken from cornmeal-agar cultures, while in the second and third experiments thiamin was not active because the spores were taken from oatmeal-agar cultures.

The minimum effective concentration of lactoflavin on spores from oatmeal-agar cultures, under the conditions of these experiments, was about 100 gamma per cc., that concentration giving nearly 100 per cent germination while a 50 gamma per cc. solution gave practically no germination. Concentrations of thiamin, nicotinic acid, carotene, ascorbic acid, and several of the amino acids ranging from 0.1 to 500 gamma per cc., with and without sucrose, caused no germination of spores cultured on oatmeal agar. At the same time a concentration of 100 gamma per cc. of any one of these substances, with or without sucrose, would induce 50 to 100 per cent germination of spores cultured on cornmeal agar. These results were repeated again and again and held true for *Colletotrichum gloeosporioides* strains from both the avocado and the papaya.

Boron in concentrations ranging from 0.001 to 10 gamma per cc. was tested for its effect on the germination of spores grown on oatmeal agar and in no instance was any germination detected. Also, synthetic lactoflavin was found to be just as active as the natural product. This would more or less rule out an interpretation that the effectiveness of the lactoflavin preparation was due to the presence of boron as an impurity. A pH effect of lactoflavin is ruled out because malic and several other acids failed to effect germination of these oatmeal-cultured spores. The stimulation of the germination of cornmeal-cultured spores by malic acid might be a pH effect, but

that cannot explain the action of carotene, a-tocopherol, and a number of amino acids with free NH_2 groups. Also indole(3)acetic acid was not effective.

SPORES OF *PENICILLIUM ITALICUM*

Lactoflavin, thiamin, nicotinic acid, ascorbic acid, malic acid, carotene, and a-tocopherol were all found to be inactive in inducing the germination of *Penicillium* spores taken directly from an orange. These spores, however, were induced to germinate by addition of either leucine, cystine, cysteine hydrochloride, valine, tryptophane, and glutamic acid hydrochloride. Since amino acids with a free NH_2 group as well as the hydrochlorides were active, it appears that the limiting factor in this case is nitrogen in the amino form.

The addition of sucrose to the amino acid enhanced germination while the addition of lactoflavin inhibited it materially. Just why *Colletotrichum* spores need lactoflavin to germinate and *Penicillium* spore germination is inhibited by it is not known. It finds a parallel in the work of SCHOPFER (7), in which it was found that while many kinds of molds need B_1 to grow, *Rhizopus* is inhibited by it.

Summary

1. Lactoflavin (natural product) and ascorbic acid were both highly effective in inducing germination of papaya pollen. Synthetic lactoflavin was only about half as active as the natural product.

2. The natural preparation of lactoflavin was found to contain boron, and boron was found to induce pollen germination. From these results it is concluded that the action of the natural preparation of lactoflavin may be the result of a combined effect of lactoflavin and boron.

3. Nicotinic acid, indole(3)acetic acid, and the hydrochlorides of several amino acids were slightly active in inducing germination of pollen, but the action of these substances appeared to be a pH effect.

4. Thiamin, nicotinic acid, ascorbic acid, carotene, lactoflavin, and a number of other substances induced germination of *Colletotrichum gloeosporioides* spores grown on cornmeal agar, but only lactoflavin induced germination of spores grown on oatmeal agar.

5. Synthetic lactoflavin was just as active in inducing germination

of *Colletotrichum gloeosporioides* spores cultured on oatmeal agar as was the natural lactoflavin preparation.

6. Amino acids induced germination of *Penicillium italicum* spores, while addition of lactoflavin to the amino acids inhibited germination materially.

The writer is indebted to MR. H. E. STEVENS, plant pathologist of the U.S.D.A., Orlando, Florida, for many helpful suggestions during the course of the spore experiments.

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PHOTOPERIODIC RESPONSES OF DILL, A VERY SENSITIVE LONG DAY PLANT¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 501

KARL C. HAMNER AND AUBREY W. NAYLOR²

(WITH THREE FIGURES)

In the literature published since the discovery of photoperiodism in 1920 (1), the responses of many plants to photoperiod, whether long day, short day, or day neutral, are presented. The fact that a plant is listed as long day, or as short day, may not of itself be sufficient information on which to base the selection of any variety for critical experimentation. All short day plants do not respond with equal rapidity to short photoperiods, nor do they exhibit identical responses at different temperatures (5). The same is true for long day plants. Some varieties may be photoperiodically sensitive at one range of temperature and day neutral at another.

In previous papers (2, 3) it has been pointed out that attempts at integration of the effects of photoperiod on the sexually reproductive responses of plants should take into account the several stages in development of flower, fruit, and seed. *Xanthium pennsylvanicum* (3) will remain vegetative indefinitely if grown continuously under conditions of long photoperiod; if exposed to photoperiods which result in the initiation of floral primordia, these primordia develop into flowers and fruits under a wide range of photoperiod, even under continuous light. On the other hand, potato (4) will initiate floral primordia even under conditions of continuous darkness, but conditions of long photoperiod are favorable for the development of these primordia into flowers and fruits. There are so many other interrelations between phases of vegetative and sexually reproductive development and environmental conditions that at present any generalization should be made with great caution. A critical comparison of the responses of several plants to a variety of environmen-

¹ This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

² Agent, Fruit and Vegetable Crops and Diseases, U.S. Department of Agriculture.

tal conditions is necessary before such generalizations can be made, and as yet sufficient information is not available to do this.

In connection with the experiments on *Xanthium*, it was desired to investigate the responses of some long day plant and to compare the two types. Many long day plants were investigated in a preliminary survey, including dill, aster (two species), *Scabiosa*, *Lepidium* sp., *Sisymbrium* sp., beet (biennial), wheat, and timothy, all of which have been reported as long day plants. Of all these, dill (*Anethum graveolens* Linn.) proved the most sensitive. The work reported here deals with some of its responses.

The seeds used throughout this work were from commercially available supplies and germinated well. They were planted in flats on a well lighted greenhouse bench under conditions of short (9-hour) photoperiod and transferred to $3\frac{1}{2}$ -inch pots after the seedlings had developed from five to ten expanded leaves. As here used, the term long photoperiod refers to an exposure consisting of the normal daylight prevailing at any particular season of the year, supplemented when necessary by Mazda light of never less than 60 foot-candles intensity, giving a photoperiod of 18–19 hours' duration, alternating with dark periods of 6 or 5 hours' duration. Short photoperiod refers to exposure to 9-hour photoperiods alternating with 15-hour dark periods.

Investigation

In the spring of 1938, several hundred seedlings of dill were grown in the greenhouse under conditions of short photoperiod. About 2 per cent of the young plants developed seed stalks in a short time and were discarded. Many of the remaining plants were kept in the greenhouse all summer until the spring of 1939, being exposed throughout this period to conditions of 9-hour photoperiods. Except for the few plants which flowered shortly after planting, all remained strictly vegetative and in the rosette condition. During the spring, summer, and fall there were relatively wide variations in greenhouse temperatures, at times ranging as low as 50° F. and at others as high as 100° to 110° F. From our stock of seed, at least 98 per cent of the plants were strictly long day and remained vegetative indefinitely under conditions of 9-hour photoperiods, even under wide ranges of temperature.

In preliminary work it was found that dill plants placed on long photoperiod (18–19 hours) flowered within 5 weeks, even though the plants were very young and possessed only three to four expanded leaves. Plants which received ten long photoperiods and were subsequently grown under conditions of short photoperiod flowered approximately as soon as comparable plants maintained continuously on long photoperiod. To supplement these preliminary results, the following experiments were carried out.

On January 7, fifty strictly vegetative plants were transferred from conditions of short photoperiod to conditions of long photoperiod and each day thereafter five plants were returned to conditions of short photoperiod. Thus some plants were exposed to one long photoperiod only, still others to two long photoperiods, and so on, some plants receiving a total of ten long photoperiods. All plants which had been subjected to four or more long photoperiods produced a flowering stalk and flowered when returned to and maintained on short photoperiod. One of the plants receiving three long photoperiods flowered while the other four plants did not. None of those plants receiving but one or two long photoperiods flowered. These latter plants were continued on short photoperiod until March 15, at which time some of them showed some stem elongation although flower primordia could not be distinguished. Evidently dill plants may be photoperiodically induced by exposure to four long photoperiods.

Another experiment was carried out in a slightly different manner. Eighty-eight vegetative plants were selected from among those on short photoperiod on February 25. Seventy-two of them were transferred to conditions of continuous illumination while sixteen were continued on short photoperiod to serve as controls. The plants on continuous illumination were exposed to natural daylight for 9 hours, and to more than 60 foot candles of light from a Mazda lamp for the remaining 15 hours of each day. After 36 hours of such continuous illumination, sixteen plants were returned to and maintained on short photoperiod. Sixteen more plants were returned to short photoperiod after 60 hours' exposure, sixteen more after 84 hours, sixteen after 108 hours, and sixteen after 132 hours. The results are summarized in table 1. It may be possible that some of the plants

receiving 36 or 60 hours of continuous illumination would have had floral primordia if they had been harvested at a later date, since all of them showed some stem elongation, but the plants were harvested on March 28 because some of them had just reached the flowering stage on that date and maximum differences could be noted between the various treatments (fig. 1).

TABLE 1

EFFECT OF CONTINUOUS ILLUMINATION OF VARYING DURATION UPON STEM ELONGATION AND FLORAL INITIATION AND DEVELOPMENT IN DILL. BEFORE AND AFTER PERIODS OF CONTINUOUS ILLUMINATION, ALL PLANTS WERE MAINTAINED ON SHORT PHOTOPERIODS. VARIOUS TREATMENTS STARTED FEBRUARY 25; OBSERVATIONS MADE MARCH 28

	NUMBER OF HOURS OF CONTINUOUS ILLUMINATION USED IN ANY ONE TREATMENT					CONTROLS CONTINUED ON 9 HOUR PHOTO- PERIOD
	132	108	84	60	36	
Average length (in cm.) of stem, based on 16 plants	57.3	38.9	19.1	19.0	8.1	3.3
Floral condition based on 16 plants	16 with macro- scopic flowers	16 with macro- scopic flowers	14 with macro- scopic flowers; 2 vege- tative	6 with in- flores- cence primor- dia; 10 vege- tative	2 with in- flores- cence primor- dia; 14 vege- tative	16 vege- tative

Perhaps the most striking differences between the various treatments are found in the relationship between the number of hours of continuous light the plants received and the amount of stem elongation. Up to 132 hours of treatment, the amount of stem elongation is almost directly proportional to the number of hours of light (with the exception of the 60-hour treatment which has a value somewhat high).

Experiments were conducted to determine whether or not, in the case of dill, the perception of the photoperiodic stimulus was received by the leaves. In a representative experiment, six pots of plants were placed in each of two wooden boxes of such depth that the

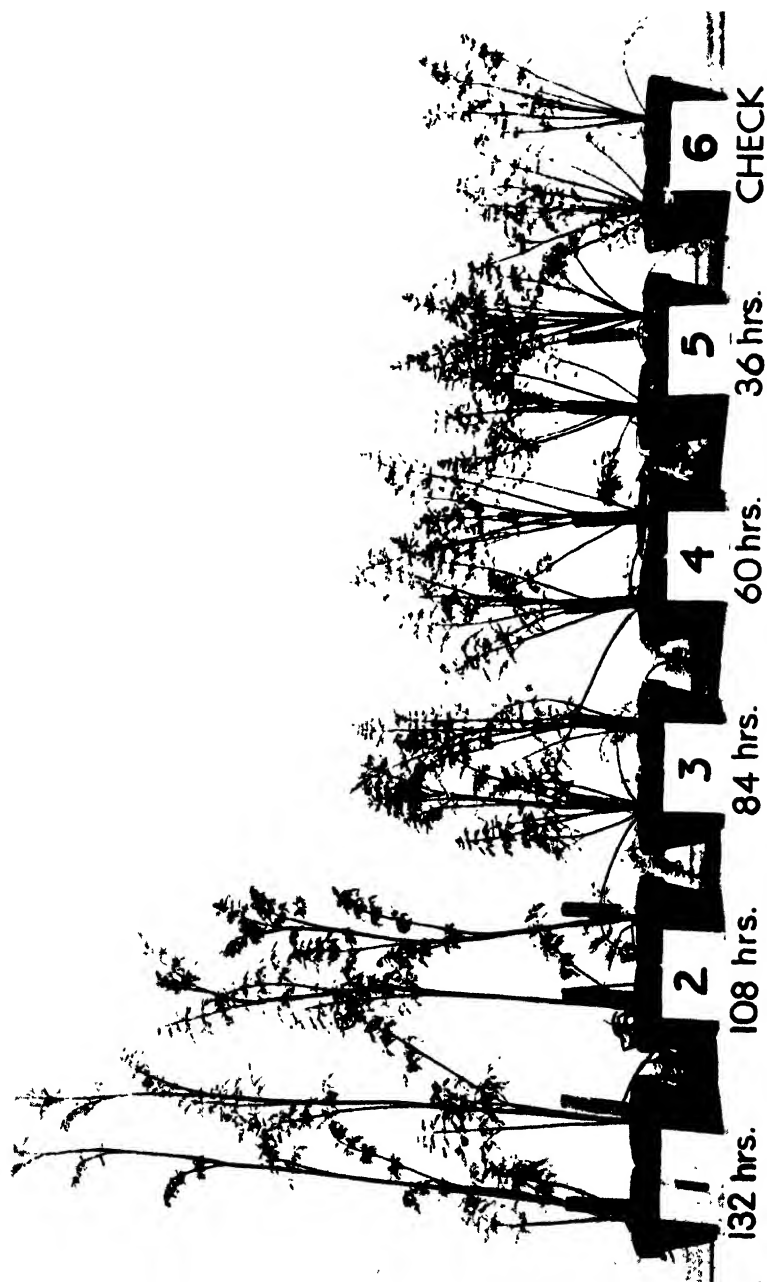


FIG. 1.—Response to continuous illumination for varying lengths of time: 1–5, beginning February 25, plants received continuous illumination in excess of 9 hours as indicated by numbers; 6, controls maintained on 9-hour photoperiod only. Before and after the indicated periods of continuous illumination, plants of 1–5 were maintained on 9-hour photoperiods. Photograph taken March 28.

leaves of the plants partially extended up out of the open top of the box. These boxes were painted black on the inside and were light-tight except for the top, which was open. Deep notches were made



FIG. 2.—Apparatus used to subject entire plants or parts of plants to same or different photoperiods at same time. *A*, showing slit in box, covered by cardboard tacked in place, and plant in position; *B*, outside view showing same assembly; *C*, control without inverted top and box in place to give short photoperiod to all but single leaf which remains exposed to light.

in the sides of one box and one leaf from each plant extended out of the box through the notch (fig. 2). After each leaf was in position, a piece of notched cardboard was placed over the notch in the box so that the petiole of the leaf extended through both notches. The

notch in the cardboard was placed at right angles to the notch in the box and the cardboard was then tacked in place. The small space next to the petiole where light might still enter the box was sealed with a mixture of modeling clay and castor oil. Thus all the leaves of each plant, with the exception of the one, were inside. The other



FIG. 3.—Effect of exposure of one leaf to long photoperiod while remainder of plant received short photoperiod. *A*, control, all leaves exposed to short photoperiod only; *B*, one leaf of each plant received a long photoperiod (January 14 to February 25).

box contained control plants and all the leaves of these plants were inside. The two boxes were placed under conditions of long photoperiod, and each night at 5:00 P.M. supplementary boxes were inverted over those containing the plants, so that no light reached any of the leaves of the various plants except the one leaf of each plant extending outside as described. At 8:00 A.M. the supplementary boxes were removed. Thus one leaf on each of six plants was exposed to conditions of long photoperiod while the remainder of the

plant was exposed to conditions of short photoperiod. At the same time six other comparable control plants had all their leaves exposed to short photoperiod only. This experiment was repeated in its entirety three times.

In every case, all plants having one leaf exposed to long photoperiod flowered within 6 weeks after start of the treatment. None of the control plants on short photoperiod flowered even though the experiment was continued for several months (fig. 3). Thus in dill, which is a long day plant, exposure of a single leaf to long day is sufficient to induce the entire plant to flower. In this respect dill somewhat resembles *Xanthium*, a short day plant, which will flower provided but a single leaf on a given plant is exposed to short photoperiod.

A number of other experiments were conducted, designed to determine the critical day length for dill. These experiments demonstrated that vegetative plants remain vegetative under ordinary greenhouse conditions at photoperiods less than 11 hours' duration and always flower at photoperiods in excess of 14 hours. Perhaps this lack of a sharp critical period in dill may be attributed to lack of genetic uniformity.

Under the conditions here described, dill is a long day plant which responds very rapidly to long photoperiod by coming into bloom. It has a short induction period, is easily grown under ordinary greenhouse conditions, and if subjected to short photoperiods will not be induced to flower by temperature variations ordinarily encountered in greenhouse work.

Summary

1. Approximately 2 per cent of the seedlings from the commercial dill seed employed flowered soon after germination if grown on 9-hour photoperiods, while the remainder continued in a rosette condition for 11 months when grown in the greenhouse at the same photoperiod.

2. Vegetative plants transferred from conditions of short to those of long photoperiod were induced to flower after exposure to as few as four long photoperiods.

3. If vegetative plants which have been maintained on short

photoperiod are transferred to continuous light for a period up to a total of 132 hours and then returned to short photoperiod, the rate of subsequent stem elongation is roughly proportional to the number of hours of continuous light received.

4. The critical photoperiod of dill, under ordinary greenhouse conditions, is between 11 and 14 hours.

5. Exposure of a single leaf to long photoperiod, although the remainder of the plant is kept on short photoperiod, is sufficient to induce the entire plant to flower.

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DEVELOPMENT OF MEGAGAMETOPHYTE IN ERYTHRONIUM ALBIDUM¹

D. C. COOPER

(WITH SIXTEEN FIGURES)

Introduction

When it appeared that the development of the megagametophyte in *Lilium* (4, 5, 6) and *Tulipa* (3) is similar to that in *Fritillaria* (1, 2), material of *Erythronium albidum* Nutt., another member of the tribe Tulipeae of the Liliaceae, was collected for examination and comparison. SCHAFFNER (13) had found that the egg is removed from the macrospore mother cell by three divisions rather than by four, as has been described later for the genera first mentioned.

The material was collected from plants growing in an area of open woods on the campus of the University of Wisconsin. Ovaries from buds of various ages, as well as from open and withered flowers, were cut transversely into small pieces and either fixed in Carnoy's solution (3 parts 95 per cent alcohol, 1 part glacial acetic acid) or dipped in that solution and then placed in Karpechenko's modification of Navashin's fluid. Satisfactory preparations were obtained by both methods. The ovaries were sectioned transversely at 15 to 18 μ , stained in dilute Delafield's haematoxylin, and counterstained with safranin.

Observations

The primary archesporial cell of *Erythronium albidum* functions as the macrospore mother cell. This cell is approximately three times as long as broad at the time of the first meiotic equatorial plate, and is somewhat flattened longitudinally. The heterotypic spindle lies in the mid-portion of the cell, its axis coinciding with the longitudinal axis (fig. 1). The spindle is completely surrounded by a dense layer of cytoplasm. The remaining cytoplasm of the cell is finely

¹ Papers from the department of botany and the department of genetics (no. 238), Agricultural Experiment Station, University of Wisconsin. Published with the approval of the director of the station.

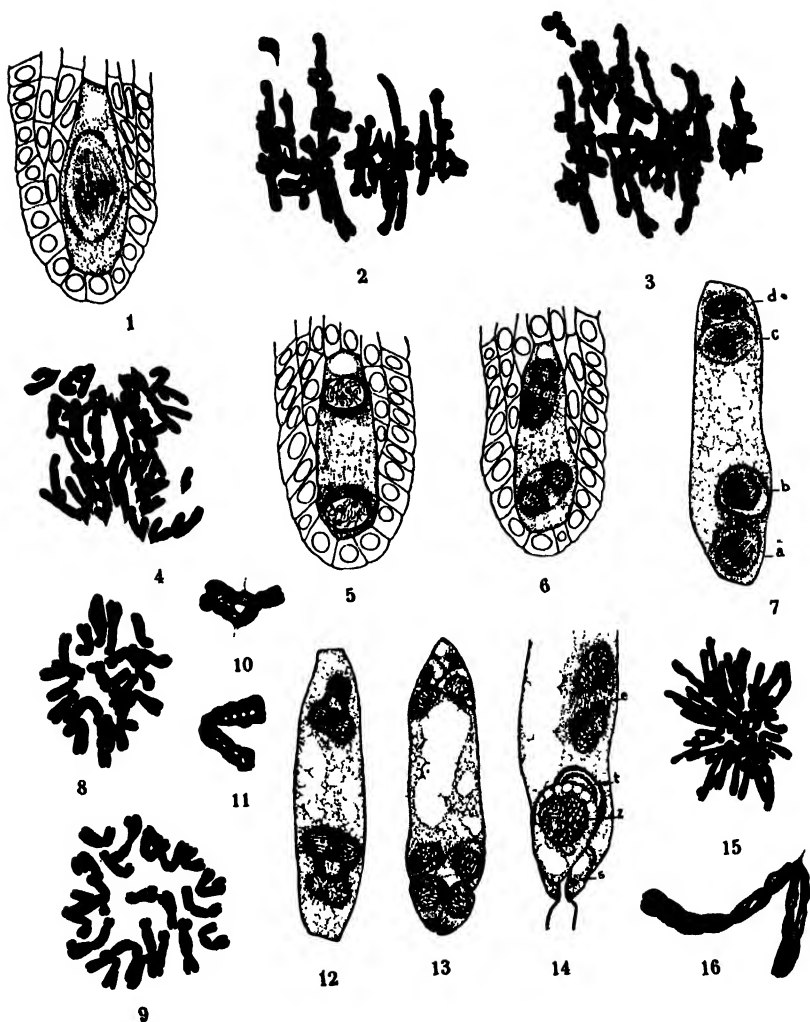
vacuolate, except that the chalazal region is occupied by a large vacuole.

Twenty-two pairs of chromosomes are present at diakinesis and on the heterotypic equatorial plate (figs. 2, 3). Anaphase figures show 22 chromosomes passing to each pole (fig. 4). The chromosomes vary in size and shape and in the position of the spindle fiber attachment. The two nuclei formed as a result of the heterotypic division are located one at each end of the mother cell. They pass into a prolonged resting stage and the heterotypic spindle disintegrates, the central region of the cell becoming more or less vacuolate. During this interphase period a zone of dense cytoplasm surrounds each of the nuclei (fig. 5).

The spindles of the homoeotypic division lie at oblique angles to the longitudinal axis of the cell. As a result of this division four nuclei are formed (fig. 6). The homoeotypic spindles are more or less persistent. In the interim between this and the third and last division, the cell grows and elongates to such an extent that it is fully four times as long as broad, and the cytoplasm in its mid-region becomes highly vacuolate.

Three of the four nuclei, the two at the micropylar end of the cell (fig. 7*a*, *b*) and the one at the chalazal end nearest the central vacuolated region (fig. 7*c*), undergo typical mitoses. Twenty-two chromosomes are present on each equatorial plate. Figures 8 and 9 are polar views of the chromosomes on equatorial plates located, respectively, in positions *c* and *a* (fig. 7). The nucleus nearest the chalazal end of the sac (fig. 7*d*) enters upon a more or less abortive division. Masses or aggregations of chromatic material, rather than definite chromosomes, appear on the spindle. Portions of this material pass irregularly to each pole and two irregularly shaped nuclei are formed simultaneously with the development of normal nuclei in other portions of the megagametophyte. Abortive divisions of the chalazal nucleus of a similar character have been figured and described (12, 11, 5, 6) for various species of *Lilium*.

Each spindle, during the third and last division in the megagametophyte, is surrounded by a dense layer of cytoplasm (fig. 7). Remnants of the spindles of the preceding division persist between the respective dense layers. The four nuclei at each end of the mega-



FIGS. 1-16.—Fig. 1, nucellus; macrospore mother cell at equatorial plate stage; $\times 175$. Figs. 2, 3, heterotypic equatorial plates with 22 pairs of chromosomes; $\times 830$. Fig. 4, heterotypic anaphase; $\times 830$. Fig. 5, nucellus; 2-nucleate megagametophyte; $\times 175$. Fig. 6, nucellus; 4-nucleate megagametophyte with persistent homoeotypic spindles; $\times 175$. Fig. 7, equatorial plates; last (third) nuclear division in megagametophyte; $\times 175$. Fig. 8, polar view of haploid equatorial plate, position c; $\times 830$. Fig. 9, same, position a; $\times 830$. Figs. 10, 11, chromosomes from haploid equatorial plates showing four chromonemata; $\times 1600$. Fig. 12, 8-nucleate megagametophyte showing cell plate formation; $\times 175$. Fig. 13, mature megagametophyte; $\times 175$. Fig. 14, late stage in fertilization showing zygote (a), dividing primary endosperm nucleus (e), disintegrating pollen tube (t), and synergids (s); $\times 175$. Fig. 15, polar view of diploid equatorial plate with 44 chromosomes; $\times 830$. Fig. 16, chromosome from diploid equatorial plate with four chromonemata; $\times 1600$.

gametophyte formed as a result of the final division are connected by two prominent spindles and by portions of a less prominent one (fig. 12), as described by SCHAFFNER (13). The synergids and egg at the micropylar end and the antipodal cells at the chalazal end are delimited as a result of cell plate formation across the spindles of the last division, as well as across the persistent spindles of the preceding division. The last division was taking place in ovaries taken from open flowers. In some instances pollen tubes were found in the region of the micropyle at the time the nuclei of the megagametophyte were in the third division.

A typical 8-nucleate, 7-celled megagametophyte is formed (fig. 13). SCHAFFNER described an 8-nucleate megagametophyte in which the egg apparatus was not well organized. GUERIN'S (8) description of the mature megagametophyte in *Erythronium dens canis* is similar to that herein described for *E. albidum* (9).

The definite organization of the megagametophyte persists for a short time only. If fertilization does not occur, the membranes delimiting the cells disintegrate and the eight nuclei lie free within the space in the nucellus formerly occupied by the gametophyte. Such aborting megagametophytes are present in many of the ovules in an ovary wherein some of the adjacent ovules have been fertilized. The polar nuclei and those of the egg apparatus increase greatly in size before disintegrating.

Fertilization occurs in the manner typical of most angiosperms. One male gamete nucleus unites with the egg nucleus; the other unites with the two polar nuclei. A late stage in fertilization is shown in figure 14, in which the fusing nuclei in the egg (*z*) are plainly evident. The collapsed pollen tube (*t*) lies beneath the zygote and the synergids (*s*) are in course of disintegration. The latter cells have become highly vacuolate and their nuclei are much shrunken. The primary endosperm nucleus (*e*) is in a late stage of the first division following fertilization.

The zygote, and at later stages the young embryo, remains at the micropylar end of the endosperm. No evidence has been found that either the zygote or the young embryo comes to lie free within the endosperm.

Discussion

SCHAFFNER (13) reported a haploid chromosome number in both *Erythronium albidum* and *E. americanum* as being 12, although his figure 68 of a homocotypic equatorial plate seems to show 22. In the present study, 22 chromosome pairs were clearly seen at diakinesis and on the heterotypic equatorial plate. Polar views of equatorial plates at the time of the third division in the megagametophyte showing 22 chromosomes (figs. 8, 9) and similar views of dividing nucellar nuclei with 44 chromosomes (fig. 15) confirm this number.

The chromosomes of dividing gametophytic nuclei (figs. 8, 9) fall into two types, based upon the region of spindle fiber attachment. Each chromosome of one type is V-shaped with the spindle fiber attachment region at or near its middle, whereas each one of the second type is a long shaft with a short rounded lobe beyond the attachment region. There are 8 chromosomes of the former type and 14 of the latter. Those of each type vary among themselves in length.

The equatorial plate chromosomes of dividing gametophytic nuclei are shorter and thicker than those at a similar stage in the adjacent nucellar tissue (fig. 15). Each chromosome on either a haploid (figs. 10, 11) or a diploid (fig. 16) equatorial plate consists of four chromonemata twisted about one another in pairs. This type of chromosome structure has been described in a number of plants which have large chromosomes (7, 9, 10, 14, and others).

The presence of the haploid number of chromosomes on one of the chalazal spindles (fig. 7c) of the last nuclear division in the formation of the megagametophyte, and the fact that the megagametophyte is removed from the spore mother cell by three rather than by four divisions, justify the reference of megagametophyte development in *Erythronium albidum* to the "Adoxa" type.

Summary

1. The primary archesporial cell of *Erythronium albidum* functions as a macrospore mother cell which develops directly into the megagametophyte.

2. In consequence of the two meiotic divisions followed by a single mitotic division and this by cell division, an 8-nucleate, 7-celled megagametophyte is formed.

3. The last mitotic division in the formation of the megagametophyte occurs just before fertilization.
4. Cell division is by means of cell plates.
5. The chromosome number is $n = 22$, $2n = 44$.
6. Eight V-shaped chromosomes with median spindle fiber attachments and 14 rodlike chromosomes with subterminal spindle fiber attachments are present in gametophytic nuclei.

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EFFECT OF CERTAIN GROWTH SUBSTANCES ON INFLORESCENCES OF DATES

ROY W. NIXON¹ AND F. E. GARDNER²

(WITH ONE FIGURE)

Varying degrees of fruit development have been induced in a number of plant species by the use of growth substances (1, 2). Attempts in 1938 to influence the course of development of date ovaries by means of these compounds were chiefly unsuccessful. A response of other portions of the inflorescence, however, appears to warrant brief mention.

Seedless fruits of *Phoenix dactylifera* often develop when pollination is prevented by bagging. As compared with pollinated seed-containing fruit, the unpollinated seedless fruit is usually smaller, somewhat different in shape, develops more slowly, and the occasional fruit that eventually ripens is generally rather fibrous and lacking in quality, although apparently of a sugar content comparable on a dry basis with the pollinated fruits (3). It was thought that treatment of flowers with growth substances might stimulate parthenocarpic fruits to a development more comparable with that of pollinated dates, and that, applied in conjunction with pollen, it might affect the set or the time of fruit ripening.

Indoleacetic, indolebutyric, and naphthaleneacetic acids were applied, alone and in combination with pollen, both before and after the application of the latter. The highest concentration used was 1.0 per cent in lanolin paste. Aqueous solutions between 0.1 and 0.01 per cent were applied as a spray, with further dilutions of naphthaleneacetic acid down to 0.001 per cent. Naphthaleneacetic acid was also applied as a dust mixed with talc, in concentrations from 1.0 to 0.01 per cent.

The higher concentrations of indoleacetic and indolebutyric acids not only failed to stimulate development of parthenocarpic fruits but resulted in the shedding of practically all flowers within a few weeks.

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In the lower concentrations there was less shedding of flowers, but the resulting fruits were apparently no different from those without

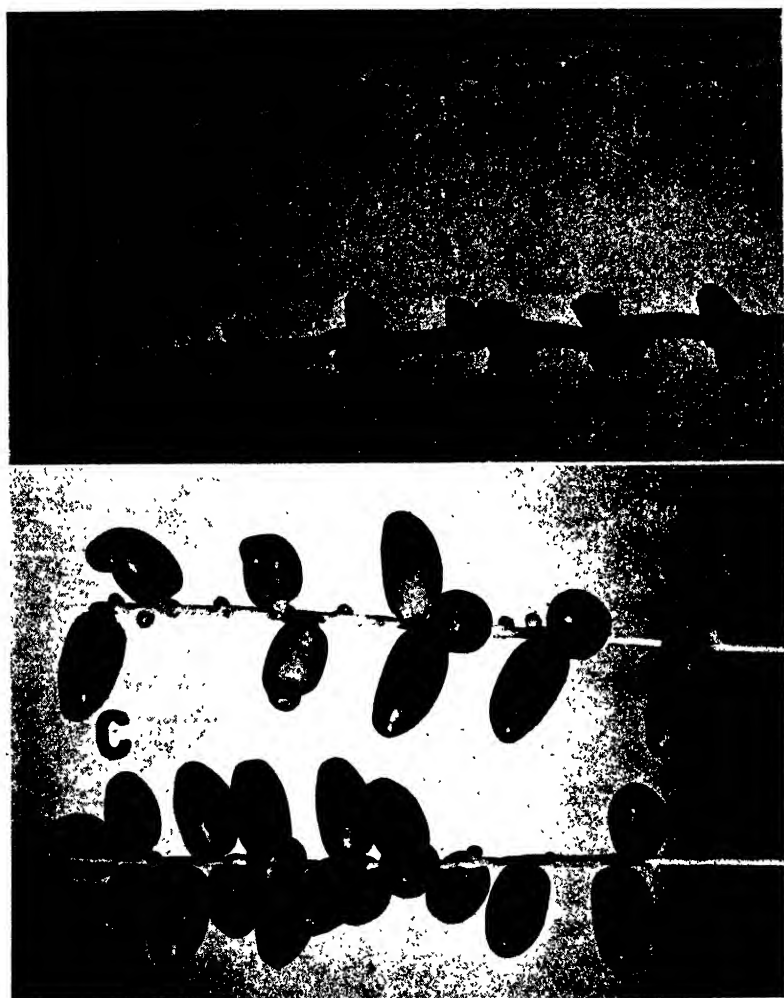


FIG. 1.—Effect of growth promoting substances of inflorescences of dates

growth substance treatment. Naphthaleneacetic acid in dust form was ineffective. In lanolin paste and in the higher concentrations of aqueous solutions, however, naphthaleneacetic acid inhibited development of the carpels, which eventually became dry and spongy,

but at the same time caused the enveloping perianths to persist and even to show apparent enlargement in some instances.

Figure 1B shows the effect of 1 per cent naphthaleneacetic acid in lanolin paste applied to date flowers of the Thoory variety on March 30, 1938. When cut on November 29 this strand with its persisting perianths was still alive and normally colored, except where the tip carrying two or three perianths had withered slightly. Much earlier than this, normally pollinated dates were all past ripe, with strands dead and dry. On the same bunch, strands treated with the other two chemicals in a similar manner lost all flowers and were dead and dry early in the season (fig. 1A). The results show the arrested senescence of the perianth and strand, an effect shown to occur in certain fruit tissues from growth substance application (5).

Figure 1C shows the effect on Deglet Noor dates, khalal stage, of naphthaleneacetic acid applied as an aqueous spray about ten days after pollination, first in concentration of 0.01 per cent and six days later in concentration of 0.02 per cent, as compared with untreated pollinated dates (fig. 1D). The set was reduced about 50 per cent by this treatment. Although less marked than in the higher concentrations, there was the same tendency toward restriction of carpellary development with persistence of perianths. Most of the pollinated dates that developed after naphthaleneacetic acid treatment appeared in every way like the untreated pollinated dates on adjacent bunches. A considerable number, however, developed with varying degrees of malformation as illustrated. The nature of the malformation indicates a marked injurious effect of the acid on the succulent carpellary tissue. Apparently unaffected dates among those treated ripened a little earlier and with a higher percentage of drier fruit than occurred on the untreated bunches. This, however, cannot be attributed directly to the growth substance but rather to the consequent reduced set, for it has been observed that severe thinning produces similar results (4).

The observations on the application of growth substances to inflorescences of dates emphasize the marked influence often exerted by these compounds, particularly by naphthaleneacetic acid, in ar-

resting senescence and delaying the abscission of various plant organs.

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CURRENT LITERATURE

Fortschritte der Chemie organischer Naturstoffe. Edited by L. ZECHMEISTER. Vienna: Springer, 1938. Pp. vi+371. Figs. 41. Rm. 28.

In the field of organic chemistry, scientific progress is so rapid that, without the ever-growing volume of reviews and monographs, the flood of publications would present a bewildering complexity of results. The *Fortschritte* is apparently to be an annual, of which this is the first volume. It is sponsored by some of the best organic chemists of Europe, including, in addition to the editor, BUTANANDT, KÖGL, HAWORTH, and SPÄTH. These names guarantee the quality of the reviews.

This volume contains seven reviews. The first, by ZEMPLÉN, deals with recent methods of glucoside syntheses⁷ and describes seven methods of synthesizing glucosides: alkyl glucoside syntheses from free sugar and alcohol by chemical agents; biochemical syntheses; syntheses from acetohalogen compounds; transformations of β -glucosides into the α -form; mercury salt methods; preparation of biosides of the α -series without employment of acetohalogens; and Helferich's phenol-bioside syntheses with zinc chloride or p-toluol-sulphonic acid.

The second review, by HILDITCH, considers the component glycerides of vegetable fats. Some of the points covered are the isolation of individual triglycerides by crystallization; crystallization of halogenated fats; and quantitative studies of component glycerides. There is an important discussion of the glyceride structure of seed fats, of both liquid and solid types.

The reviews of recent advances in the chemistry of the sterols, by HEILBRON and SPRING, and of cozymase, by SCHLENK and VON EULER, are timely. Advances in these fields have been exceptionally rapid, and the reviews are a revelation of the insight into the structure of complicated organic cellular agents which organic chemistry has given us. Nucleic acid chemistry is reviewed by BREDERECK, the main consideration being given to the structure of the nucleosides, nucleotides, and polynucleotides. A review of chlorophyll, by STOLL and WIEDEMANN, occupies nearly 100 pages, and carries a literature list of over 400 references. It is a thorough going, masterful summary of current knowledge of this pigment and its derivatives.

The final review, by KRATKY and MARK, considers several topics, but mainly the use of physical methods in the investigation of natural substances, as regards the form and size of molecules, in the dispersed state. The determination of active groups, cryoscopy, vapor pressure depression, dialysis, osmometry, ultracentrifugation, and free diffusion are considered in the first section; in the second, the application of roentgenography to the study of structure is presented; the final section deals especially with the use of x-rays in the study of protein structure.

It is not possible here to do justice to this volume; it must be examined to grasp its significance. It is certainly one of the best groups of reviews on biochemistry issued in recent years. The series will be indispensable for all libraries and for all who are actively engaged in biochemical research.—C. A. SHULL.

The World of Plant Life. By C. J. HYLANDER. New York: Macmillan Co., 1939. Pp. xxiv+722. Illustrated. \$7.50.

This survey was planned and written primarily for the layman as a "card of introduction to the outstanding personalities in the plant world. . . . The natural interests of the average American, at home and vacationing in our summer and winter playgrounds, has been the criterion for the inclusion of material." It is secondarily designed for students as "an introduction to an alive, fascinating world which is often ignored in laboratory study"; and as a picture of the whole plant kingdom for amateur or professional specialists. The reviewer feels that all three aims have been fairly well achieved. Popularization with strict accuracy is not easy for most botanists, even in their special fields. Such treatment for the whole plant kingdom is an ambitious undertaking, and this one is deserving of commendation.

The author, recognizing the difficulty of his task, approached it from a taxonomic point of view. Upon the foundation of phylum, order, family, and tribe he has arranged interesting and accurate facts of form, function, ecology, distribution, and use of about 2000 of the more common native and introduced plants of continental United States. The emphasis is upon plants of the vacation centers of the northeast, Florida, California, and the southwest, and spectacular plants perhaps receive disproportionate attention. These features are understandable in a work of this kind. About three-fourths of the volume is devoted to angiosperms. The enjoyment of the book by both laymen and botanists is markedly increased by the numerous and beautiful photographic illustrations and clean and accurate pen-and-ink drawings. There is a good index, a well selected reference list, and a 47-page check list giving the scientific classification and common name of all species mentioned in the text. The book should rightly stimulate the interest of the general public in plants.—C. E. OLMSTED.

The Physiology of Plants. By WILLIAM SEIFRIZ. New York: Wiley & Sons, 1938. Pp. xvii+315. Figs. 95. \$3.50.

A number of texts on plant physiology have appeared recently. This work by SEIFRIZ is characterized by its brevity and elementary nature. The entire field is covered in 26 chapters, in 305 text pages. Following a brief introduction, which defines the aims, scope, and historical background of the subject, the author gives early consideration to the colloidal state, soil, protoplasm, and the organized plant cell.

A fairly logical order of presentation follows, with water relations: osmosis, sap movement, use, loss, and storage of water the main consideration, in preparation for a study of the major processes. Acidity, salt requirements, and per-

meability follow in order, and then photosynthesis, respiration, carbon and nitrogen cycles, movement and storage of foods, nature of foods, and the products derived from them, such as cellulose, wall substances, etc., and other plant products. The later chapters discuss growth and hormones, plant cultures, reproduction, movements, plant associations, environment, and a chapter on the living and nonliving state. While the chapters are short, yet a great deal of information is provided. General principles are stressed, and the work will no doubt prove popular because it is not too technical nor too detailed.

The reviewer feels that the brevity of this text almost reaches the point of leaving inaccurate impressions; taken as a whole, the work corrects some of these impressions. On page 244, for instance, it is said that "water culture as a laboratory technique was known a quarter of a century ago." The implication seems to be that it began then; but the author has previously mentioned in another connection the early laboratory use of solution cultures of SACHS and KNOP, which are more than 60 years old. The experiment cited from HALES' work is called a root-pressure experiment, although the root is lifting a column of mercury under the influence of transpiration by the top of the tree. DUTROCHET's discovery of osmotic movement of water was published originally in 1826, not in 1837. One may wonder also why the date 1838 is chosen as the starting point of plant physiology as a science.

Any slight faults which may be present are not likely to detract greatly from the usefulness of the book. There has been need of a work suitable for beginning students, and this text has caught something of the qualifications necessary for such a purpose.—C. A. SHULL.

Statistical Methods. By GEORGE W. SNEDECOR. Ames, Iowa: Collegiate Press, 1938. Pp. xiii+388. Illustrated. \$3.75.

Those who may have found other works on statistical methods forbidding should examine this excellent exposition of the subject. It is addressed particularly to the novice, and the reviewer is impressed with the nontechnical language in which the various procedures are explained. The author has demonstrated that discussion of the mathematical principles involved in statistical studies is not necessarily uninteresting and unintelligible to the beginner. The more readily mastered principles and techniques are treated first, and new concepts are introduced one at a time; in this way progress is gradual, and each new step easy to take. Here is a manual of statistics which apparently can be used without excessive study of mathematics. It necessarily covers much the same ground as the well known work by R. A. FISHER, but the approach is somewhat simpler and less technical.

The first seven chapters deal with the simplest problems the investigator is likely to encounter: statistical study of attributes; comparative measurement of individuals; sampling of normal populations; statistical comparison of two groups; short cut methods and approximations; linear regression; and correlations. The succeeding chapters cover the more complicated cases: large sample

theory; multiple degrees of freedom; variance; covariance; multiple regression; curvilinear regression; individual degrees of freedom; and binomial and Poisson distribution.

Tables are introduced where they are needed, not in an appendix; and numerous examples from actual experience are used as problems to illustrate the processes of statistical analysis. This use of concrete material makes the presentation far more effective than would the mere discussion of principles and methods, however thorough.

The book is recommended as a constant companion of those who are attempting to establish the validity of conclusions from mass study of responses. Most investigators sooner or later find that statistics are necessary to the solution of their problems; moreover, if the principles are mastered, more frequent use would naturally be made of these methods of testing results. The plane of experimental work would be raised considerably if the statistical methods outlined in this volume were more generally employed. No better work is available for the beginner.—C. A. SHULL.

Hardy Bulbs, Vol. III: *Liliaceae*. By CHARLES HERVEY GREY. New York: Dutton & Co., 1938. Pp. x+664. Figs. 82. \$11.00.

This volume is the third in a series in which an attempt has been made to describe—in a manner useful to gardeners and botanists alike—all bulbous, tuberous, and rhizomatous monocotyledons of garden value that are capable of cultivation in the open or in cool greenhouses, and to give information as to site, cultivation, and propagation.

Volume III embraces those plants which are classed as Liliaceous according to the system of ENGLER and PRANTL. The genera, numbering 110, are listed alphabetically. A brief generic description is given and this is followed by a description of the species which are considered as hardy and worthy of cultivation. The book is essentially a reference work and contains a vast amount of valuable information. It includes 19 color plates and 63 black and white drawings.—J. M. BEAL.

Experiments in Plant Physiology. By WALTER E. LOOMIS and CHARLES A. SHULL. New York: McGraw-Hill, 1939. Pp. xiv+213. \$2.00.

This book contains directions for 167 elementary and intermediate experiments in plant physiology, for the most part selected from the more comprehensive text, *Methods in Plant Physiology*, published in 1937 by the same authors. The work covers practically every phase of the subject, and more experiments are presented than can be covered in the usual course. Some of the experiments can be performed with hand-made, inexpensive apparatus, while others require more elaborate and expensive equipment. The contents of the book are thus flexible so that it can be used in longer or shorter courses and by institutions that have little or much money for laboratory equipment.

The directions are admirably presented, in simple, clear, and concise lan-

guage so that the student may easily follow them. The book fills a long-felt need and is a distinct contribution to the subject of elementary plant physiology.—E. C. MILLER.

Hilfsbuch für das Sammeln und Präparieren der niederen Kryptogamen. 2d ed.

By Dr. GUSTAV LINDAU; revised by Dr. O. C. SCHMIDT. Berlin: Borntraeger, 1938. Pp. vii+93. Rm. 3.60.

This revised booklet, which includes citations of a few 1938 United States publications, should be of value to botany students in general even though the bibliography is composed mainly of German publications.

While necessarily brief in its specialized part, which includes collecting directions for the various groups of thallophytes and bryophytes, this manual discusses in some detail many important or interesting genera groups and genera. Suggestions for collecting plants, and directions for drying them and preventing insect infestation are given; formulae for various preserving solutions are added where needed. The volume will be of greatest value to amateur collectors of Continental Europe, but may appeal to a wider, international audience.—P. D. VOTH.

Principles of Paleobotany. By WILLIAM C. DARRAH. Leiden, Holland: Chronica Botanica Co., 1939. Pp. 239. Plates 5.

Few universities offer a course in paleobotany, and most students who desire to become acquainted with this subject must depend upon literature and whatever museum collections are available. An introductory book in paleobotany is therefore a much desired addition to botanical literature. DARRAH's arrangement of the subject is rather satisfactory. He gives the reader an idea of literature and technique before entering into the discussion of fossil plant phyla. As a whole, the morphological, ecological, and geological features are as well developed as the small space of the book permits. The beginner will find it interesting reading. The mature reader may differ with DARRAH on his valuation of events in paleobotanical history. It must also be regretted that a book which appeals primarily to the beginner should contain so few illustrations.—A. C. NOÉ.

Cotton. By HARRY BATES BROWN. 2d ed. New York: McGraw-Hill Book Co., 1938. Pp. xiii+592. Figs. 140.

This book brings up to date the material presented in a previous volume by the same author some twelve years earlier. There has been added considerable new material dealing with the morphology of the cotton plant, its culture, and the economic handling of the crop.—E. J. KRAUS.

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